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Determination of Immune-Boosting Trace Elements In Selected Fruit Seeds

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ABSTRACT

The deluge of processed food in the world has led to an incalculable number of health problems and increased mortality. Consequently, a healthy and long life has become a gem that every human being is chasing in this modern society. Due to the lacuna of information about a healthy lifestyle, scientists have shifted their focal point to carry out painstaking research in the field of nutrition such as trace elements. Reviewing prior literature, it is evident that controlled intake of trace elements leads to healthy skin and hair, aids in blood sugar control, prevents osteoporosis, and is involved in protein synthesis, treatment of hypertension, and inflammatory conditions. Intake of large amounts of the elements brings about health problems lowering the body's immunity. This study analyzed trace elements of selected fruit seeds (watermelon seeds, orange seeds, pawpaw seeds, avocado seeds) commonly consumed in Kendu Bay, Homa Bay County, and club 36, Nairobi County to determine the moisture content and levels of trace elements in the seeds then compare the concentrations of the trace elements (Zn, Cu, Fe, Mg, Mn) in the various selected fruit seeds with the limits recommended by WHO and FAO. The wet digestion method was used to digest them after which they were analyzed by atomic absorption spectrophotometer. The average moisture content in orange, pawpaw, watermelon, and avocado seeds was 50.26%, 87.38, 72.19%, and 63.75 respectively. The levels of the trace elements were analyzed using atomic absorption spectrophotometer (AAS). The results indicated that all the trace elements positively correlated with copper, giving a strong correlation coefficient (r=0.9997) with a regression equation of y=0.0238x-0.0002, while manganese had the lowest correlation (r= 0.9617). Pawpaw seeds from Kendu Bay had the highest levels of zinc (0.6020±0.5794mg/kg), magnesium (4.2270±0.1479mg/kg), and manganese (0.2743±0.0564mg/kg). Avocado seed from club 36 had the lowest levels of zinc (0.2102±0.0076mg/kg) and magnesium (1.6063±0.0594mg/kg). Orange seeds from club 36 recorded the highest levels of copper (0.1597±0.0005mg/kg), while pawpaw seeds from club 36 recorded the lowest levels of copper (0.1177±0.0004mg/kg). It is evident from the results that magnesium was the abundant trace mineral in all the fruit seed samples, with a concentration of 4.2270±0.1479mg/kg in pawpaw seeds from Kendu Bay. Manganese and copper recorded the lowest levels across all samples. Comparing the results with WHO/FAO permissible limits for trace minerals in food, all elements in the fruit seed samples were below and within the permissible levels. Iron contents were below the detection limit in all samples. The findings of this study will provide additional information on the immune-boosting trace elements present in the selected fruit seeds from Kendu Bay and Club 36, Nairobi. The study will be of great importance to nutritionists, consumers worldwide, and health agencies in creating awareness of the health benefits of fruit seed consumption

Keywords : Trace elements, Fruit seeds, Healthy, long life, Atomic Absorption Spectrophotometer and Analysis.

I. INTRODUCTION

Fruits have long been ingested universally by both humans and animals to satisfy their hunger and replenish their thirst. In Sub-Saharan Africa's dry regions and deserts, for example, watermelon is frequently consumed to satisfy thirst [1]. Moreover, fruits possess a complex and vast concoction of active ingredients such as antioxidants, polyphenols, and carotenoids [2]. Fruit-rich diets are widely advocated for their health-promoting qualities since it offers substantial doses of biologically active compounds that have health benefits beyond just providing fundamental nutrition [3]. Yet the majority of fruit consumers are in the dark about the optimal blend of polyphenols, flavonoids, phenolic acid, dietary fiber, carotenoids, lipids, and microelements that are densely concentrated in the seeds of fruits [4]. Little concentrations of minerals known as microelements are found in biological tissues of living things including fruit seeds. Selenium, zinc, chromium, vanadium, manganese, copper, and iron are some of these elements. In order to survive ecologically and pass on their genes to the next generation, plants must consume these nutrients which, therefore, accumulate in the plant's fruits, seeds, stems, leaves, and roots.

Trace elements can be subdivided into three categories: essential trace elements, major elements (Fe, Ca, and P), and toxic heavy metals (Pb, Hg, and Cd). Example of essential trace elements includes; Cr, Cu, Fe, Mn, Mo, Se, and Zn. These elements lead to healthy skin and hair, aid in creating deoxyribonucleic acid (DNA), blood sugar control, the building of proteins, the growth of cells, and healing damaged tissues, they are essential components of hemoglobin, growth and strength of bones, the immune system, and heart function. Brain development, biochemical reactions such as production in the liver, superoxide dismutase, which is vital in preventing oxidative stress, and pyruvate carboxylase, an essential enzyme in gluconeogenesis [5].

With the analytical method recognized as atomic absorption spectroscopy, these trace element levels can be quantified. This method is founded on the idea that when atoms absorb light, they gain energy and transition from the ground state to the excited state [6]. The analyte absorbs light at a specific wavelength, and as a result, the concentration of the analyte may be determined from the absorbed light. Standard solutions of the same metals are examined for their concentration in order to actualize the concentrations of the trace elements of focus. The calibration curve, which applies the Beer-Lambert law to connect the analyte's concentration to its absorbance, is constructed using the collected data on concentration and absorbance.

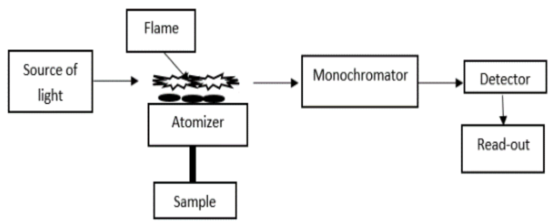

Figure 1 : A diagram of AAS instrumentation

II. Statement of the problem

Regrettably, the development of food technology and the dearth of sufficient food to feed the enormous global population have contributed to the manufacturing of processed food products. These processed foods have worsened health problems and mortality because they result in to rise in the risk and prevalence of obesity and other nutrient-related chronic illnesses [7]. The aforesaid factors make it crucial to find strategies to enhance health and ensure a long life for people.

Among the main sources of the micronutrients and microelements that are essential for promoting a long and healthy human existence are the seeds of fruits. Unsurprisingly, many only consume the fresh, juicy parts of edible fruits and throw away the seeds [8]. Thus, scholars should cease this general public's ignorance about the health advantages of fruit seeds.

III. Main Objective

To determine and quantify the immune-boosting trace elements in selected fruit seeds.

3.1: Specific Objectives

i. To determine the essential trace elements, present in pawpaw, orange, avocado, and watermelon seeds.
ii. To determine the concentration levels of the trace elements in the selected seed samples.
iii. To compare levels of the immune-boosting trace elements analyzed across the selected fruit seeds in each study area.
iv. To compare the essential trace elements of the sample seeds with the established Recommended Dietary Allowance stipulated by WHO and KEBS.
v. To determine the moisture content of the fruit seed samples.

3.2: Justification of The Study

Every person hopes to live a long and healthy life. Consuming a healthy meal is among the things that might help you realize this desire. However, the abundance of processed food in the global food industry denies this desire. These processed food products have resulted in poor health and the short lifespan of the average human which have drawn the attention of scientists, politicians, and members of the public. This study sought to educate people throughout the world about the significance of the area of nutrition by examining the amounts of various trace components. Health organizations and cosmetics industries can also use the data to improve their nutritional practices and embody fruit seeds in their cosmetic products respectively [2].

IV. Materials and Methods

4.1. Study Area

The study was carried out at Club 36 and Kendu Bay. These study areas were selected due to the availability of samples, scientific studies carried out in the area, and high consumption of fruits in those areas.

Club 36 is located along Dorobo road near Nairobi primary and Women's hall 20 (Box) in Nairobi. It is between latitude -1.2830374° S and longitude 36.8110191° E. It is a small business center where food is the primary commodity sold. It is the main eatery for the most university of Nairobi students and workers in the nearby offices.

Figure 2 : Map showing the location of the Club 36 study area.

Kendu Bay town is a bay in Kenya located on the shore of Lake Victoria along Katito-Homa Bay road. It is a town in East Rachuonyo Division, Rachuonyo North Subcounty, and Homa Bay County in Western Kenya [9]. It lies between latitude -0.3684°S and longitude 34.6497°E. The town has a population of 6,064 [10] and mainly has loamy soil with mineral contents of K, Mg, Ca, Fe, and Mn, as reported by Oduor (2014). The fruits sold in Kendu Bay town are mainly grown in that area. Table 3.1 shows the list of towns near Kendu Bay:

Figure 3 : Map showing the location of Kendu Bay town market study area.

4.2. Project design

To realize the desired objectives of the study, it was divided into three primary phases. They are sample collection, digestion of the sample, and laboratory analysis.

Table 1 : Towns Near Kendu Bay Town

Town	Approximate distance(km)	The direction of the towns from Kendu Bay town
Oyugis	17km	South East
Homa Bay	28km	South West
Kisumu	32km	North East

4.3: Collection of the sample

Prior to the study, fieldwork was carried out. The data obtained helped to determine the season of the year to sample, the locations of the study area, and the type of fruits to be sampled. Four fruits were selected, that is, watermelon, papaya, orange, and avocado. Two samples of each fruit were purchased taking necessary precautions from Kendu Bay town market and Club 36 and tagged according to the type of fruit and location. They were collected in a synthetic carrier bag and transported in cooler boxes containing ice cubes to the department of chemistry analytical laboratory at the University of Nairobi. The fruits were then washed using tap water, their seed extracted, and oven-dried.

4.4: Preparation and Acid Digestion of The Sample

The seed sample was dried in the oven at 105 ºC for 12 hours. They were then crushed in a cleaned, dry pestle and mortar to obtain a fine powder. The powder of each sample was stored in a polyethylene bag which was labeled with the details of the sample while they await digestion.

Figure 4: Oven drying of the samples.

Metals in the fruits are bonded to the matrix of the fruit making it impossible to analyze their concentrations. Sample digestion is done to convert the seed samples to a suitable form for chemical analysis. One of the techniques of digesting is acid digestion using aqua regia. Aqua regia solution is a chemical reagent composed of nitric acid and hydrochloric acid at a ratio of 1:3.

Before digestion, the samples were dried again for an hour. 1.00g of each sample was weighed and transferred into different clean, dry 250ml conical flasks in which 32 ml of aqua regia (was made by mixing 65 % w/w HCl and 37 % w/w HNO_3) was added. The solution was heated on a hot mantle plate for 2 hours. The sample solutions were then cooled in a desiccator, filtered in 100ml volumetric flasks, and made the volume to the mark. The prepared samples were then stored for further analysis.

Figure 5 : Digestion of the samples.

4.5: Preparation of The Stock solutions

1.00g of an analytical grade of different metals to be analyzed was prepared by dissolving their salts in distilled water and acid in the ratio of 1:1 (40ml distilled water, 40ml acid) in a 1L volumetric flask, and the solution was diluted to the mark with distilled water to give 1000ppm of the metal ions.

Five other metal solutions were prepared by diluting the stock solution with distilled water using $C_1V_1 = C_2V_2$, where C_1V_1 is the initial volume, and C_2V_2 is the final concentration and volume. The absorbance vs concentration of the five solutions was measured, and a calibration curve was plotted. A blank solution and the standard solution were prepared for each working solution. The aliquots of the metal solutions prepared were placed in storage bottles ready for analysis.

Figure 6 : Photo of the AAS used to analyze the samples.

4.6: Sample Analysis

After preparing the standard solutions of each trace element, the samples and the standards were introduced in the AAS (Shimadzu Model AA-6300), and the absorbance and concentration readings were obtained. Table 2 below shows the working conditions of the AAS.

Distilled water was used as blank at intervals to trace contamination sources and make sure the reading returned to its initial setting before measuring the absorbance of the standards and the samples.

V. Results and Discussion

5.1: Introduction

One of the objectives of the study was to determine the moisture content and the levels of magnesium, zinc, manganese, copper, and iron ions in the duplicate seed samples. The concentration and absorbance of the standards (Zn, Cu, Fe, Mn, and Mg) analyzed by the AAS were used to plot the calibration curves of the metals. These calibration curves were used to realize the levels of the trace elements which were presented as mean and standard deviation. This section presents, interprets, and discusses the data of the seed samples sampled from Club 36 and Kendu Bay town market.

5.2: Trace Elements Levels

The levels of all trace elements under study were analyzed using the AAS (Shimadzu Model AA-6300) for all the selected samples. Levels of the trace elements analyzed (the analysis was done in duplicates) were Mg, Zn, Mn, Cu, and Fe. The standard deviation and average values for each trace element were calculated using M.S. Excel.

5.2.1: Zinc

From table 3 and 4, the level of zinc was found to 0.1928±0.0351, 0.2102±0.0076, 0.2909±0.0046, 0.2957±0.0075, 0.3336±0.0600, 0.4098±0.0218, 0.4506±0.0698, and 0.6020±0.5794 in avocado (Kendu Bay), avocado (Club 36), orange (Club 36), orange (Kendu Bay), watermelon (Kendu Bay), pawpaw (Club 36), watermelon (Kendu Bay), and pawpaw (Kendu Bay) respectively. The level of zinc was highest in paw seeds from Kendu Bay (0.6020±0.5794) and lowest (0.1928±0.035) in avocado seeds from the same study area. All the fruit seed samples in the present study were below the recommended levels since the recommended dietary allowance for Zn by the USA National Institute of Health (NIH) is 8 to 11 mg/day for 19 to 70 years adult men and women [11].

5.2.2: Manganese

Manganese is vital in digestion and bone growth, as Oduor (2014) reported. Pawpaw seeds from Kendu Bay had the highest levels of manganese in the range of (0.2743±0.0564mg/kg), followed by pawpaw seeds from

Table 2 : The working conditions for AAS

	Zn	Fe	Cu	Mg	Mn
Method	AAS	AAS	AAS	AAS	AAS
Lamp current	8Ma	12mA	6mA	8Ma	10mA
Flame	Acetylene	Acetylene	Acetylene	Acetylene	Acetylene
Oxidant/support	Air	Air	Air	Air	Air
Wavelength	213.9nm	248.3nm	324.8nm	285.2nm	279.5nm
Slit width	1.0nm	0.2nm	0.7nm	0.7nm	0.2nm
Burner height	7mm	9mm	7mm	7mm	7mm

Fuel gas flow rate	2.0L/min	2.2L/min	1.8L/min	1.8L/min	2.0L/min
Support gas flow rate	15.0L/min	15.0L/min	15.0L/min	15.0L/min	15.0L/min

club 36, Nairobi, which ranged between (0.2244±0.0042mg/kg). Orange seeds from Kendu Bay recorded the least amount of manganese being (0.0907±0.0507mg/kg) (from Tables 3 and 4). Its concentration levels ranged from 0.0907±0.0507 to 0.2743±0.0564 mg/kg, which was within the recommended daily allowance of 0.2 to 0.4mg/kg by WHO.

Table 3: Average concentration of the analyzed trace elements in pawpaw and avocado seeds.

	Avocado seed		Pawpaw seeds	
	Kendu Bay	Club 36	Kendu Bay	Club 36
Zn	0.1928±0.0351	0.2102±0.0076	0.6020±0.5794	0.4098±0.0218
Cu	0.1181±0.0010	0.1272±0.0079	0.2743±0.0564	0.2244±0.0042
Mn	0.1127±0.0236	0.1191±0.0005	0.1364±0.0564	0.1177±0.0004
Mg	1.9938±0.1313	1.6063±0.0594	4.2270±0.1479	4.0194±0.0726
Fe	BDL	BDL	BDL	BDL

Table 4: Average concentration of the analyzed trace elements in watermelon and orange seeds.

	Watermelon seeds		Orange seeds	
	Kendu Bay	Club 36	Kendu Bay	Club 36
Zn	0.3336±0.0600	0.4506±0.0698	0.2957±0.0075	0.2909±0.0046
Cu	0.1064±0.0039	0.1572±0.0030	0.1199±0.0093	0.1597±0.0005
Mn	0.1864±0.0349	0.1245±0.1056	0.0907±0.0507	0.1614±0.0394
Mg	1.6401±0.0566	1.7069±0.2281	3.4069±0.5095	2.8717±0.1044
Fe	BDL	BDL	BDL	BDL

5.2.3: Magnesium

Magnesium plays a role in over 300 enzyme chemical reactions in the body, such as bone development, maintaining normal blood pressure, energy production, and synthesis of essential molecules such as RNA, DNA, and proteins [9]. The level of magnesium in the fruit seed samples was found to be 4.2270±0.1479, 4.0194±0.0726, 3.4069±0.5095, 2.8717±0.1044, 1.9938±0.1313, 1.7069±0.2281, 1.6401±0.0566, and 1.6063±0.0594 in pawpaw (Kendu Bay), pawpaw (club 36), orange (Kendu Bay), orange (Club 36), avocado (Kendu Bay), watermelon (Club), watermelon (Kendu Bay), and avocado (Club 36). According to CCNFSDU, the tolerable upper limit of magnesium is 350 mg/day, and the RDA for Mg is 310mg/day [12]. The seed samples recorded lower mineral levels, as indicated in Tables 2 and 3. Such lower levels may cause adverse health conditions such as cardiovascular disease and hypokalemia [9].

5.2.4: Copper

Tables 3 and 4 show Cu concentrations in the seed samples analyzed. Orange seeds sample from Club 36 Nairobi had the highest copper amount (0.1597±0.0005mg/kg). This was closely followed by watermelon seeds from Club 36, Nairobi, with (0.1572±0.0030mg/kg). Watermelon seeds from Kendu Bay had the least copper content. The stipulated permissible Cu levels are 1.0mg/kg in food by WHO [12]; all the seed samples recorded concentrations below these limits. Cu is required for brain development, and its deficiency causes Wilson's disease. A combined diet of these fruit seeds can provide the required copper levels for the body.

5.3: Distribution of the Essential Trace Elements analyzed in the study

Trace elements varied from one fruit seed to another. This variation is influenced by the mineral composition of the soil where the fruit is grown, the weather condition of the area, the mineral composition of the water used for irrigation, agricultural practices such as the amount and type of farm fertilizer used, and also a variety of the fruit also contributes to the mineral content **(13)**.

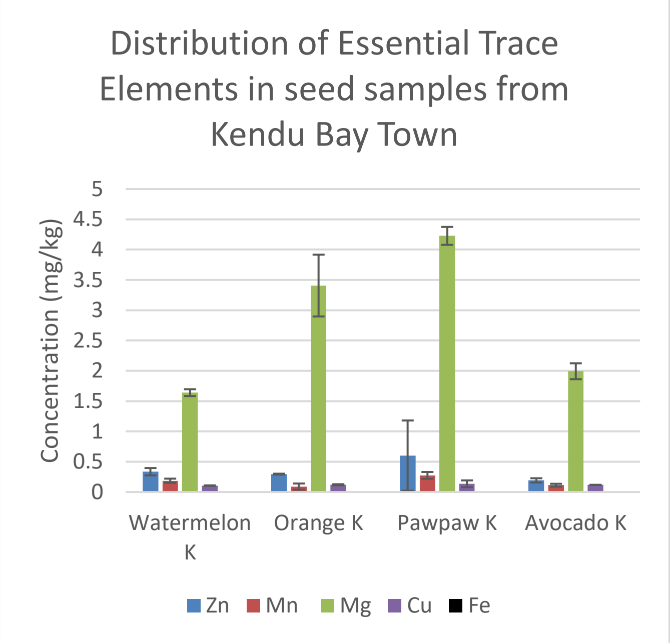

Figure 7: Distribution of Essential Trace Elements of Fruit Seeds in Kendu Bay (mg/kg). The error bars represent ±SD

Figure 7 above shows the average concentration distribution of the trace elements in the selected fruit seeds by comparing trace element levels in each seed sample collected in Kendu Bay. Magnesium was the most abundant trace element in all the samples, while copper was the least. The following sequence of the essential trace elements was observed: Mg>Zn>Mn>Cu>Fe.

Like the Kendu Bay samples, magnesium recorded the highest concentrations across all samples, while copper and manganese recorded the least. The trend of the trace elements analyzed in the seed samples was observed in the following sequence: Mg>Zn>Mn>Cu>Fe (figure 8). This study sequence significantly differed from the trend of (Fe>Mn>Zn>Mg>Cu) noted in Jacob et al. 2017 research study. Morais et al., 2017 recorded a different trend of Mg>Cu>Fe>Zn>Mn in avocado, papaya, and watermelon seeds, where magnesium had the highest value range compared to Mn and Zn and Fe. Yilmaz and Karaman (2017) also recorded a sequence of Mg>Zn>Fe>Mn in the study of mineral contents for orange seed fiber crackers.

5.4: Regression Lines

The readings of each standard solution were recorded, and the correlation coefficient and a regression equation were calculated using M.S. Excel. Correlation values range from +1 to -1, where a value of 1 shows a perfect positive correlation, -1 indicates a perfect negative correlation, and 0 indicates no linear relationship exists.

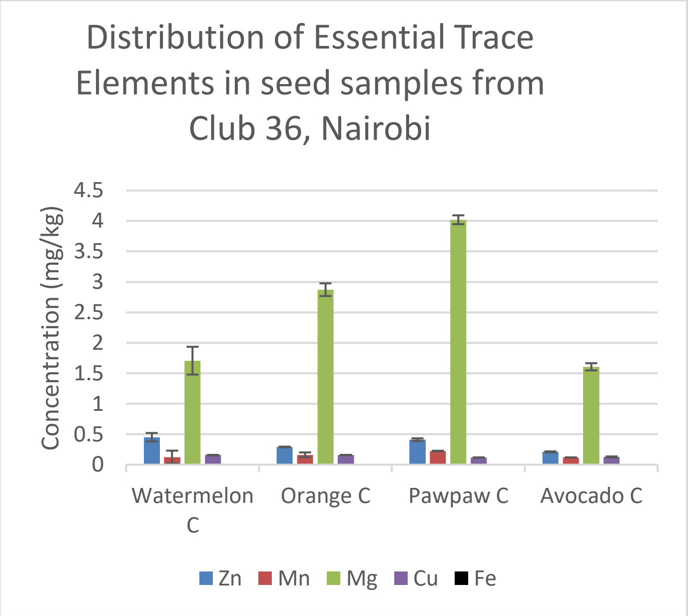

Figure 8: Distribution of Essential Trace Elements of Fruit Seeds in Club 36, Nairobi (mg/kg). The error bars represent± SD

Table 5: Correlation Coefficient of the Essential Trace Elements

Parameter	Instrument	r 2	Regression equation

Zn	AAS	0.9983	y=0.0764x-0.0005
Fe	AAS	0.9628	y=0.0118x-0.0011
Cu	AAS	0.9997	y=0.0238x-0.0002
Mn	AAS	0.9617	y=0.0378x-0.0025
Mg	AAS	0.9732	y=0.2468x-0.0032

The r^2 values in the results were within the linear range hence a positive correlation. The values agreed with the acceptable values of best fit. Copper had the highest correlation coefficient(r^2=0.9997) with a regression equation of y=0.0238x-0.0002 while manganese had the lowest correlation (r^2= 0.9617). The r^2 values for other trace elements were as shown in Table 5.

5.4: Moisture content

This study employed the oven-dry method. This method was used by Nya et al. (2000) to compare the moisture content of seeds in two varieties of bush mango (Irvinga gabonensi). The samples were dried in the oven at 105°C overnight. 25.66g of each sample was measured and analyzed. According to a study carried out in Kendu Bay by Oduor (2014), the following formula was used to determine the moisture content of vegetables:

$$\text{Moisture content } \frac{\text{initial weight} - \text{oven dried weight}}{\text{initial weight}} \text{ x } 100$$

With reference to Tables 6 and 7, the moisture content of the seed fruit at Club 36 is 56.82%, 63.74, 72.17, and 87.43% in orange, avocado, watermelon, and pawpaw respectively while the moisture of fruits from Kendu Bay was 43.69%, 63.76%, 72.21%, and 87.33% in orange, avocado, watermelon, and pawpaw respectively.

Table 6: Moisture Content of Samples from Kendu Bay Town (wet weight basis)

Seed samples	Wet-weight (g)	Dry-weight (g)	Mass of water(g)	Moisture content (%)
Orange seeds	25.66	14.54	11.12	43.69
Pawpaw seeds	25.66	3.25	22.41	87.33
Watermelon seeds	25.66	7.13	18.53	72.21
Avocado seed	25.66	9.30	16.36	63.76

Table 7: Moisture Content of Samples from Club 36, Nairobi (wet weight basis)

Seed samples	Wet-weight (g)	Dry-weight (g)	Mass of water (g)	Moisture content (%)
Orange seeds	25.66	11.08	14.58	56.82
Pawpaw seeds	25.66	3.23	22.43	87.43
Watermelon seeds	25.66	7.14	18.52	72.17
Avocado seed	25.66	9.31	16.35	63.74

During oven drying, the papers used for drying the samples become oily, and the seeds had a pleasant smell. An indication that the fruit seeds have fats and oil and are also used as snacks.

VI. Conclusion and Recommendation

6.1: Conclusion.

This study determined the concentration of five trace elements and moisture content by employing the AAS and the oven drying technique respectively. It was concluded that the fruits contained a reasonable concentration of trace elements and moisture content. This assessment provided a vivid image of some of the compositions of avocado, orange, watermelon, and pawpaw seeds. The aim of the study was to determine the selected trace elements in the selected fruits. In addition, the data obtained can also help to protect the health condition of people when these seeds are incorporated into nutrition, that is, increased consumption of these fruit seeds may help in boosting the body's immunity. The variation in the moisture content was attributed to the different amounts of rainfall received in these areas and the fruit plant intake of water.

6.2: Recommendations

The recommendations deduced from this study include:

1) A follow-up study is recommended to correlate the trace element levels in the selected fruit seeds to the levels of the same elements in the soils where they are cultivated. A questionnaire survey is recommended to precede the latter to trace the fruits' sources.
2) Other analytical techniques such as X-ray Fluorescence Spectrometry, Inductively Coupled Plasma-Mass Spectrometry, and Inductively Coupled Plasma Optical Emission Spectroscopy should be employed in future studies.
3) More studies should analyze other essential trace elements in edible fruit seeds nationally and worldwide.
4) Health agencies such as WHO should put in place the proper mitigation measures that should be taken to improve the levels of the essential elements in the seeds where their levels are below and above the acceptable limits.
5) Further studies should be done by analyzing the essential trace elements in other types of seeds apart from those analyzed in the present study.

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