THE ANTINUTRIENTS PROPERTIES AND SOME HEAVY METALS CONTENTS OF *PERSEA AMERICANA* (AVOCADO PEAR) SEEDS

BY

EHIWERE OISEIDE MERRY

(BMS1702539)

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY OF BASIC MEDICAL SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF UNIVERSITY OF BENIN, BENIN CITY.

JANUARY, 2023

CERTIFICATION

We the undersigned hereby certify that, EHIWERE OISEIDE MERRY (BMS1702539) carried out this work, in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the reward of Bachelors of science Degree (B.Sc.) in Medical Biochemistry.

Signed:

…….. ……..

Dr. N.B. Aguebor-Ogie Date

……. ..……

Prof. (Mrs.) A.A Omonkhua Date

……..... ……

External Examiner Date

DEDICATION

I dedicate this project to Almighty God, whose love and mercies has kept me and is the source of my strength and achievement, and to my parents for their support all my life.

ACKNOWLEDGEMENTS

My foremost Gratitude is to my Heavenly Father, who is ever loving and faithful to his words.

My heartfelt Gratitude goes to my Loving Parents Mr. and Mrs. Ehiwere for their love, prayers, moral and financial support and discipline. I couldn't have had better parents and I love you both deeply.

To my amazing Siblings, Bright, Peace and Obehi, your unending support financially and encouragements is never forgettable.

I am profoundly grateful to my project supervisor, Dr. N.B. Aguebor-Ogie, who I respect so much. I am truly honored and grateful for your nice support, patience and intellectual guidance in seeing to the successful completion of this project work. I will not fail to acknowledge the Head of Department and lecturers of the Department of Medical Biochemistry who have equipped me with the knowledge of research.

Special thanks to my friends Cindy, Mercy, Ose, Lysander and those who I did not mention. I love you guys. God bless you all.

TABLE OF CONTENTS

Abstract

Research has been ongoing on many plant materials, especially those discarded as waste, to exploit their nutritional and antinutritional properties. Generally, plant parts (seeds, leaves, bark, fruits, stems) contain bioactive agents with medicinal properties but require proper assessment. Avocado *(P. americana*) seeds are often discarded after taking the pulp of the fruit. Until recent times, Researchers have identified a wealth of therapeutic compounds in the husk of avocado seeds which are normally discarded. The present study was aimed at determining the level of anti-nutrients and possibly heavy metals in the seed of Avocado. The seeds were removed from the pulp, washed, dried, cut into smaller pieces and allow to air dry completely and then pulverised. The seed powder of *P. americana* was analysed to determine its mineral contents and some antinutritional properties (oxalate and phytate). The quantitative analysis of these parameters showed that the anti-nutritive components are Phytate (1.07%) and oxalate (6.16%) respectively. The mineral analysis revealed zinc (3.415 mg/kg ±0.145), Lead (0.035 mg/kg ±0.005), iron (2.775 mg/kg ± 0.105), Nickel (0.115 mg/kg ±0.005), Copper(0.265 mg/kg ±0.015), Cobalt (0.14 mg/kg ±0.01), Chromium (0.08 mg/kg ±0) respectively. This study, therefore revealed that *P. americana* seeds have a low level of oxalate and phytate contents while there were low levels of some heavy metals as seen in the result. In conclusion, the seeds *of P. Americana* were found to be a beneficial potential therapeutic agent.

CHAPTER ONE

1.0 INTRODUCTION

1.0 Background of study

There is a global trend towards industrial fruit processing and following such processes byproducts are normally discarded. However, these byproducts can cause environmental problems such as increased production of wastes, insects and rodents. Thus, investigations are being made to know the benefits of these byproducts as sources of medicinal products or food supplements (Ramos *et al.,* 2004).

Avocado (*Persea americana*) is a tropical fruit belonging to the Lauraceae family. It is native to Mexico and Central and South America. Avocado (*Persea americana*) is widely consumed due to its high nutrition content as well as its therapeutic properties. Persea americana has been applied ethnomedically, most especially in some African countries. It is believed to have aphrodisiac properties (Asha *et al.,* 2009). In recent years, research has focused on various parts of the plants. The fruit's skin is used as a vermifuge and a treatment for dysentery. The aqueous extract from the leaves is used to correct hypertension and irregular heartbeat (Odugbemi, 2006). The leaves, roasted and ground seeds are used for diarrhea and dysentery. Using avocado seed oils can help treat skin breakouts (Swisher, 1988), wound healing (Nayak *et al.,*2008) and hepatoprotection (Kawagishi *et al.,* 2001). For diabetics, the edible flesh serves as a high-energy source because it contains little sugar (Justina et al, 2016). It contains vitamins, minerals, and healthy fats which help prevent disease and keep your body in good working condition.

Avocado seeds are one of the under-utilized non-edible parts of the fruit (Bahru *et al.,* 2019). Normally, the seed is usually tossed aside as residues during the processing of the pulp causing serious environmental pollution (Figueroa *et al.,* 2018). Efficient waste byproduct management would be advantageous from an economic and environmental viewpoint (Arajo *et al.,* 2020). Avocado seeds make up a significant portion (13%–17%) of the avocado fruit and are abundant in a variety of bioactive and functional substances, such as polysaccharides, proteins, lipids, minerals, and vitamins (Melgar *et al.,* 2018). Avocado seeds are rich in phenolics, flavonoids, and condensed tannins, among other bioactive substances. The bioactivities of these extracts have been studied, including their ability to reduce hyperglycemia (Tremocoldi *et al.,* 2018), prevent cancer (Lara-Marquez *et al.,* 2020), reduce inflammation (Dabas *et al.,* 2013), and reduce hypercholesterolemia (Uchenna *et al.,* 2019). Numerous conventional uses as dermatological applications include anti-oxidant (Soledad *et al.,* 2021), anti-microbial (Villarreal-Lara *et al.,* 2019), and anti-neurogenerative properties. They don't contain any hazardous or dangerous substances, making them a good natural source of biologically active components for the food, pharmaceutical, and cosmetic industries (Tremocoldi *et al.,* 2018).

Additionally, the avocado seed is a good source of carbohydrates, protein, fat and some mineral elements such as Phosphorus (P), Calcium (Ca), Potassium (K), Iron (Fe), Sodium (Na), Zinc (Zn), Copper (Cu), Cobalt (Co), and Lead (Pb) which makes them a good option for both human and animal nutrition to make up for micronutrient deficiencies (Justina *et al.,* 2016). It also contains as well as high concentration of anti-nutritional factors such as phytate, oxalate and cyanogenic glycosides making the seed appear potentially toxic (Nwaogu *et al.,* 2008).

In Nigeria, the powered seed is often mixed with soups, pap and pudding in the belief that it is useful in the management of chronic hypertension,

1.1 AIMS AND OBJECTIVES

Research has shown that the avocado seed is a good source of carbohydrates, protein, fat and some mineral elements as well as other antinutritional factors such as phytate, oxalate and cyanogenic glycosides making the seed appear potentially toxic (Nwaogu *et al.,* 2008). This study is, therefore, aimed at analysing the antinutrients properties and some heavy metals contents of *Persea Americana* (Avocado Pear) seeds as a step towards establishing purposeful utilization of the seed

CHAPTER TWO

LITERATURE REVIEW

2.0 Avocado Pear (*Persea americana*)

The fruit of *Persea americana* (*P. Americana)* commonly known as avocado is also known as alligator pear; It belongs to the Lauraceae family, having its origin in south-central Mexico which is easily adaptable to tropical regions (Morton, 1987). *P. americana* is called "*Ube bekee*" or "*Ube oyibo*" in Igbo, "*Orumwu*" in bini, "*Eban Mbakara*" in Efik.

Persea americana is a leafy tree up to 20 feet high with a trunk diameter of 60cm, with alternate and elliptical leaves and small flowers. The trees are shallow-rooted and have poor water uptake. Roots are coarse and will raise pavement with time. Branch damage results in the release of dulcitol, a white, powdered sugar, at scars (Maureen, 2014). The flowers (6-7 mm long) can be both protogynous (stigma responsive before pollen shed) and protandrous (po llen shed before stigma receptive) in the same individual. The flowers are either receptive to pollen in the morning or release pollen in the following afternoon (type A), or are receptive to pollen in the afternoon, and release pollen in the following morning (type B) (Thomas, 2018). Self-pollination (of the same flower) is prevented in this way. However, flowers on an entire tree are in different stages, and self-pollination does occur occasionally (Ashman *et al.,* 2004). The flowers are primarily pollinated by bees. Cross-pollination between types A and B maximizes

production. Except for when it's cold outside, pollination of the flowers by bees and hoverflies is normally successful (Thomas, 2018).

Fig 2.1: Fruits of P. *Americana* (Fasasi, 2018)

Fig 2.2: Tree of P. *Americana* (Fabio, 2016)

The fruit is sometimes known as an alligator pear or butter pear that has an olive-green peel and thick pale yellow pulp. It has a nutlike flavor and flesh that is buttery in its consistency. Compared to seedlings, which take 8–20 years to reach fruition, grafted plants often do so in about one to two years. Fruits weigh between 100 and 1000 grams and are 7-20 cm long and 7-10 cm in diameter. They have a large central seed that is 5–6.4 cm long (Dowling, 1987). Despite being categorized botanically as a berry, many people mistakenly believe it to be a drupe (Armstrong, 2000). *Persea americana* contains 20-30% fat which 93% is unsaturated. This makes them richer in fat than any other fruit except olive. The fatty acids it contains include linoleic, oleic, palmitic, stearic, linolenic, capric and myristic acids (Eduardo *et al.,* 2013).

2.1 Botanical Classification Of *Persea Americana*

Kingdom: Plantae

Phylum: Angiosperms

Division: Tracheophytes

Class: Magnoliids

Order: Laurales

Family: Lauraceae

Genus: Persea

Species: *P. Americana*

Binomial name: Persea Americana

2.2 Types of Persea *americana*

There are more than 500 avocado varieties. Some can be grown in warm winter temperate regions; others can be grown only in semi-tropical and tropical regions (Steve, 2016). But the three main varieties are:

- Hass Avocado
- Fuerte Avocado
- Pinkerton Avocado

Fig 2.3: Pinkerton Avocado (Corinne, 2015)

Fig 2.4: Fuerte Avocado (Josie, 2016)

Fig 2.5: Hass Avocado (Parker, 2022)

The Hass variety of Avocado, a hybrid, is the most popular and commercialized variety around the globe most especially in Nigeria. It originated in Guatemala. The tree is short and quite open. It yields a 200–300 g, medium-sized fruit. When ripe, the skin turns dark and purplish-black. Soft avocados are typically regarded as over-ripe and potentially rancid. When it is ripe, it yields gentle pressure. The Hass Avocados mature after two and a half years and a mature tree of Hass avocado can produce more than 1000 (200 kg) fruits under good farm management practices (Maxipharo, 2020).

Each avocado tree has both male and female parts, but they are active at opposite times of the day. Hass avocado trees are type A, meaning that they flower from February through May. When the flowers first open in the morning, they are female until they close in the afternoon. The following afternoon, they reopen as pollen-producing male flowers. So, Hass avocado trees can be said to be self-pollinated.

A study has shown that Hass avocado farming in Nigeria grows well in the southern region and areas with deep loose soil (Maxipharo, 2020). Hass avocados are available year-round like most agricultural commodities these days, but January through March is the best time of year for

flavor. It is during this time that the fruit has developed higher oil content, resulting in that buttery flavor and texture that we all love (Carolyn, 2018).

2.3 Nutritional and Health benefits of Hass Avocado

Hass avocados constitute about 73% of water, with other varieties of nutrients. It contains about 15% fat, 8% carbohydrates, and 2% protein (Adda, 2020). It has low calories and sugar compared to other fruits.

Hass avocado is very rich in fiber and a rich source of fatty acids which helps to protect the body against inflammation, cancer, diabetes and heart disease. It is also rich in micronutrients for human consumption such as polyphenols, fats, oils, vitamins (Vit. C, E, K, B_1, B_2, B_6, B_9) and minerals (P, Na, Mg, K, Fe and Zn) (Orhevba and Jinadu, 2011).

2.4 Hass Avocado Seed

The avocado contains a seed that, together with the skin, comprises about 13–17% of the total weight of the avocado. When eating the avocado, one cuts the fruit in half and removes the seed and skin to use the pulp of the avocado for consumption. The avocado seed is thus considered a waste product and is usually discarded. However, it may be of interest to the industry as a source of bioactive compounds. It has a lot of nutritional value as compared to other seeds which are stated in this review. The typical avocado seed has 95 calories, 17 grams of starch, 2 grams of sugar, 1.5 grams of protein, 0.5 grams of healthy fats, and 6 grams of fiber (Monica, 2016).

2.5 What Does A Hass Avocado Seed Contain?

Information about its composition is limited, but it does contain a good range of fatty acids (linoleic, oleic, palmitic, stearic, linolenic, capric and myristic acids), dietary fiber, carbs and a

small amount of protein (Deepti *et al.,* 2013). The carbs in an avocado seed consist mainly of starch, with its dry weight being nearly 75% starch.

Other studies described the presence of polyphenols (catechin, isocatechin, protocyanidin, flavonoids, tannins and proanthocyanidin monomerics), saponins, glucosides (D-perseit, D-α-manoheptit, D-monoheptulose, persiteol), sterols (β-sitosterol, campesterol, stigma sterol, cholesterol), the amino acid carnitine and two glucosides of abscisic acid (Vanya, 2020).

Furthermore, the seed appeared to be a rich source of mineral elements (Ca, Zn, K, Na, P, Fe, Cu, Pb and Co), Vitamins (A, B_1, B_2, B_3, C and E) and a broad range of phytochemicals. While some of the phytochemicals in an avocado seed may have antioxidant potential, others may not offer any health benefits.

The incredible fiber content combined with natural oils, vitamins and minerals leads to improved digestion, and reduced inflammation and has even been linked to preventing the development of cancer cells.

2.6 Economic importance of Heavy Metals in Hass Avocado Seed

Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing concern for ecological, evolutionary, nutritional and environmental reasons. Heavy metals can collect in various human organs and have long biological half-lives in addition to not degrading, which might have undesirable side effects (Egbenda *et al.,* 2015).

Chromium (Cr), nickel (Ni), silver (Ag), cobalt (Co), iron (Fe), copper (Cu), cadmium (Cd), zinc (Zn) and manganese (Mn) are a few examples of heavy metals (Haddad *et al.,* 2012). Plants uptake heavy metals either through absorption from deposits on the plant parts exposed to

polluted environmental media or through irrigation with contaminated water (Dorcas *et al.,* 2016).

Heavy metal uptake and bioaccumulation along the food chain raise a great threat and health challenges to humans as well as animals (Onakpa *et al.,* 2018). Heavy metals may also be referred to as trace elements as they are present in minute amounts in different environmental matrices (Tchounwou *et al.,* 2012). A lack of certain heavy metals, such as chromium, copper, iron, manganese, magnesium, and zinc, can cause deficiency disorders or syndromes since they are among the necessary elements needed by the body for biochemical and physiological processes. Although some heavy metals are useful or necessary, their overabundance can harm cells and tissues (David *et al.,* 2012).

Every bodily function depends on minerals, but because the body cannot produce them, it depends on the diet to provide adequate levels for absorption (Grembecka and Szefer 2011; Nabrzyski 2007). Bioelements by themselves do not contain energy, in contrast to macronutrients. To sustain excellent health throughout life, however, metals like copper (Cu), zinc (Zn), or iron (Fe) are crucial for vital biochemical and physiological processes (Nabrzyski 2007). They join with specific proteins to create enzymes that serve as catalysts for several bodily processes (Grembecka and Szefer 2011). Calcium (Ca), is a mineral that makes up important structural elements throughout the body and regulates nerve and muscle function. Chromium (Cr) and cobalt (Co) are also necessary for the metabolism of lipids, carbohydrates, and the production of proteins. Additionally, Co is a crucial component of vitamin B12, whereas humans only need trace amounts of Cr(III) for sugar metabolism, and nickel (Ni) has a variety of functions in hormone action, lipid metabolism, the activation of various enzymes, and the stabilization of DNA and RNA (Grembecka and Szefer 2011; Nabrzyski 2007). However, long-

term exposure to Cr(VI) compounds may result in allergic contact dermatitis or permanent eye damage. IARC classifies Cr(VI) and Ni as known human carcinogens (IARC Monographs 2012), although an excessive consumption of Co has been linked to thyroid gland damage, heart muscle damage, and overproduction of red blood cells (Grembecka and Szefer 2011).

2.7 Health benefits of Hass Avocado seed

The health benefits of avocado seeds are still insufficient scientific evidence. However, there is a growing body of data indicating the potential health-beneficial effects of avocado seeds. Although mostly performed in animals, several studies investigated the biological effects of avocado seed extracts and showed promising results. Avocado seed extracts were considered to have the following effects: anticancer, anti-inflammatory, antidiabetic, antihypertensive, hypocholesterolemic, antioxidant, dermatological uses, antimicrobial, and insecticidal (Vanya, 2020).

- Avocado seeds provide a healthy dose of monounsaturated fats which can help to lower cholesterol and prevent heart diseases!
- Avocado seeds have anti-inflammatory compounds that can prevent hypertension, arthritis, and Alzheimer's disease!
- Avocado seeds help rejuvenate and repair our overall skin tone and its underlying conditions!
- Avocado seeds help in maintaining our blood sugar levels.
- Avocado seeds are also known to have certain digestive benefits preventing conditions like diarrhea and constipation!
- Avocado seeds also help play a vital role in preventing cancers of most kinds!

- Avocado seeds possess the effect to boost your metabolism, which in turn helps you in being more active!
- Avocado seeds are rich in all types of vitamins, fibers, and nutrients, this help boosts your immunity too!

In Mexico, avocado seeds are used in traditional medicine to treat skin rashes, diarrhea, and dysentery caused by amoebas, to cure infectious processes caused by fungi and bacteria and for the treatment of asthma, high blood pressure, and rheumatism (Vanya, 2020).

We can see that avocado seed is exceptionally beneficial for our health, but like everything, the consumption must be in moderation and as part of a healthy balanced diet only.

2.8 Anti-Nutrients

According to nutrition information, high doses of avocado seeds are not suitable for our health. More recent studies also found a toxic effect, but from what level is toxic to humans, is still unknown. Some studies suggest that avocado seeds contain anti-nutrients like saponins, tannins, oxalate and phytic acid. Consuming the seed daily will lead to these anti-nutrients going into your body, as the name suggests, these chemicals reduce or block the absorption of nutrients and micronutrients from the digestive system into the bloodstream (Tina and John, 2019). This leads to minor to significant discomfort in your digestive system.

According to the Harvard T. H. Chan School of Public Health (Tina, 2019), specific interference for the antinutrients found in avocado seeds include:

Saponins: Overall reduction in nutrient absorption

Tannins: Reduced iron absorption

Oxalate: Reduced calcium absorption

Phytic acid: Reduced iron, zinc, magnesium and calcium absorption

2.8.1 Oxalate

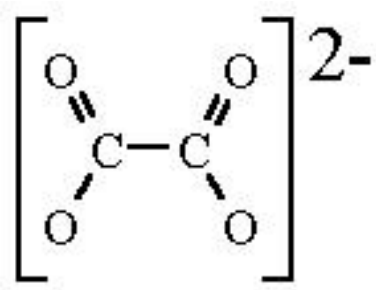

Fig 2.3: Molecular Structure of Oxalate (Diana *et al.,* 2018)

Chemical formula: $C_2O_4^{2-}$

Molar mass: 88.019 g·mol−1

Conjugate acid: Hydrogenoxalate

Oxalate (IUPAC: ethanedioate), is an anion having the chemical formula $C_2O_4^{2-.}$ This dianion has no color. Oxalate is a substance that our bodies naturally make as well as obtain from foods like spinach, almonds, potatoes, dates, etc. It is a conjugate base of oxalic acid. Oxalate is created when oxalic acid reacts with other minerals.

Following ingestion, oxalate can combine with minerals to generate compounds like calcium oxalate and iron oxalate. As a result, the body is unable to absorb and use this crucial mineral. Although it can also happen in the kidneys and other urinary tract organs, the colon is where this happens most frequently.

The majority of people then eliminate these substances through microbial oxalate metabolism in the gut or their stools or urine (Miller *et al,* 2017).

However, high oxalate diets have been connected to an increased risk of kidney stones and other health issues like osteomalacia and rickets among sensitive people.

2.8.1a MINERAL ABSORPTION MAY BE HINDERED BY OXALATE.

Oxalate can bind to minerals in the intestine and hinder the body from absorbing them, which is one of the biggest health concerns concerning it.

For instance, spinach contains a lot of calcium and oxalate, which hinders the body's ability to absorb much of the calcium (Heaney and Weaver*,* 1989).

However, it's critical to keep in mind that not all minerals in the diet will bind to oxalate.

2.8.1b REASONS TO AVOID OXALATES

Some detrimental impacts of a HIGH oxalate-rich diet include:

1. Increased Risk of Kidney Stones

Although some people are more at risk than others, estimates show that 1 in 10 people experience kidney stones. Oxalate has a higher propensity to bind to calcium and create kidney stones when levels are high.

2. Lower Absorption of Minerals

Oxalates can impede your body from receiving healthy nutrients in your digestive tract because they bond to minerals like calcium. However, they do not entirely prevent absorption, and our bodies only utilize a part of the nutrients we eat (Ben, 2020).

3. Antibiotic Interactions

Your gut breaks down some of the oxalates you consume, which lowers the amount that enters your digestive and urinary systems. However, this effect is lessened when you take antibiotics. The beneficial bacteria in our gut that absorb oxalates are reduced by antibiotics, which can enhance their activity (Ben, 2020).

Oxalates are naturally excreted by the body, but excessive amounts can be harmful. You shouldn't, however, completely cut out items containing oxalates from your diet because they frequently provide nutrition in other ways.

Making sure your diet includes a variety of fruits and vegetables in addition to high-oxalate items can help you acquire the right amount of nourishment. Opting for a healthy diet and way of life can help lessen the effects of oxalates (Dan, 2020). This comprises:

- Getting enough water will aid in your body's removal of oxalates.
- Consuming enough calcium, which helps the body break down oxalates,
- Consuming less sodium and sugar, which can increase the risk of kidney stones,

- Receiving the authorized doses of vitamin C; exceeding this amount can lead to an increase in oxalic acid production.
- Some veggies' oxalate content can be reduced by cooking them.

For patients with high urinary oxalate levels and kidney stone risk, doctors now advise a strict low-oxalate diet of fewer than 100 milligrams per day (Ran *et al.,* 2012).

2.8.2 PHYTATE

Chemical formula: C6H18O24P6

Molar mass: 660.029 g·mol−1

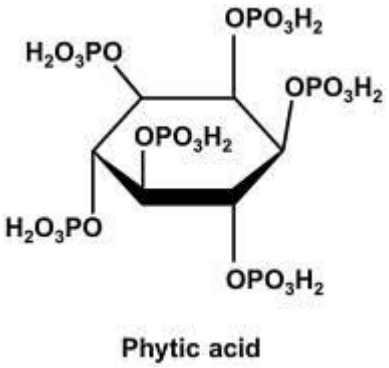

Fig 2.4: Molecular Structure of Phytate (Masashi, 2013)

Phytate also known as inositol hexaphosphate, or IP6 represents the major storage form of phosphorus (P) and inositol that plants store. (Cosgrove, 1980), and has also been suggested as storage of trace elements in plants. Phytate is then degraded and the phosphorus is released when seeds sprout. The young plant will utilize phosphorus. Between 60 and 80% of the total

phosphorus in cereals, beans, nuts, and oilseeds are phosphorus bound to inositol, primarily as phytate. In lesser quantities, roots, tubers, fruits, and berries also contain phytate.

Phytate is regarded as anti-nutritional, having detrimental effects on the bioavailability of necessary dietary minerals in monogastric animals due to a lack of enough endogenous phytate-degrading enzymes (Cosgrove, 1966; Cheryan, 1980). Iron, zinc, and calcium cannot be absorbed by phytate, which could lead to mineral deficiencies (Lydia et al, 2021).

This only relates to one particular meal, not to daily nutritional absorption as a whole. In other words, phytic acid mostly hinders your ability to absorb minerals during the meal but has little impact on following meals.

The amount of iron, zinc, and calcium you absorb from nuts, for instance, may be decreased if you snack on them in between meals, but not from the meal you consume a few hours later.

However, over time, mineral shortages may arise if you often eat foods high in phytates with your meals. For people who consume a well-balanced diet, this is rarely a problem, but in underdeveloped nations where grains and legumes are the primary sources of nourishment, it could pose a serious problem.

2.8.2a How Can Phytic Acid In Food Be Reduced?

It is not ideal to stay away from all phytic acid-containing foods because many of them are wholesome and nourishing. Additionally, because food is in short supply in many developing nations, people must rely on grains and legumes as their main dietary mainstays.

You can try a few food preparation techniques that can dramatically lower the phytic acid level of meals rather than avoiding these foods. Here are some of the most commonly used methods:

- Soaking. To lower the number of phytates in cereals and legumes, water is frequently added overnight(Gustafsson and Sandberg, 1995).
- Sprouting. Phytate breakdown results from the sprouting of seeds, grains, and legumes, sometimes referred to as germination(Weston and Deanna, 2020).
- Fermentation. Organic acids produced during fermentation encourage the breakdown of phytate. The recommended approach is lactic acid fermentation, as in the creation of sourdough (Leenhardt *et al*, 2005).

Combining these techniques can significantly lower phytate levels. For instance, boiling legumes for one hour can up to 80% lower their phytate content (Lan *et al,* 2018).

Additionally, the content of phytates is broken down (degraded) via sprouting and lactic acid fermentation (Yianna *et al,* 2022).

2.8.2b Is Phytate Harmful To Your Health?

Phytate has no health risks to people who consume a balanced diet. However, people who are susceptible to iron or zinc deficiencies should diversify their diets and not include high-phytate foods in all meals (Weston and Deanna, 2020).

For vegetarians and vegans as well as individuals who are iron deficient, this may be of particular importance. Heme iron and non-heme iron are the two forms of iron found in food. Meat and other animal products contain heme iron, whereas non-heme iron is present in plants.

Heme-iron is more effectively absorbed than non-heme iron found in diets originating from plants. Heme iron is not greatly impacted by phytate, while non-heme iron is impacted (Sandrine et al, 2008).

Additionally, even in the presence of phytate, zinc is readily absorbed from meat (Sandström *et al.,* 1989). As a result, phytate-induced mineral shortages are rarely a problem for people who eat meat.

However, when diets consist mostly of high-phytate foods while at the same time low in meat or other animal-derived products, phytate might be a serious concern.

This is especially concerning for many developing countries where a major portion of the diet consists of whole-grain cereals and legumes. The bottom line Consuming foods high in phytates, like grains, nuts, and legumes, can increase the risk of iron and zinc insufficiency.

Techniques including soaking, sprouting, and fermentation is frequently used as a defense. For people who routinely consume meat, deficits brought on by phytic acid are not a problem. Contrarily, eating foods high in phytates as part of a healthy diet provides many advantages. These advantages typically outweigh any detrimental impacts on mineral absorption (Atli, 2022).

Consumption of avocado seeds is not recommendable due to insufficient scientific evidence. However, the list of bioactive compounds that contain an avocado seed and the possible health benefits suggest otherwise. Thus, it is not said that the avocado seed is not edible, but scientific research about the safety and utility of avocado seeds (especially in humans) remains in the preliminary stages (Vanya, 2020)

CHAPTER 3

1.0 MATERIALS AND METHOD

3.1 Materials

Mature avocado fruits, Knives, Airtight bags, Masking tape, Bowl, Conical flask., Whatmanm no 1 filter paper, Oven, Magnetic stirrer, Desiccator, Water bath, Beaker, Kieldahl flask, Distilled water, 250 ml digestion tube, Heater, Funnels, 25 ml volumetric flask, Beakers, Nitric acid, Perchloric acid, Hydrochloric acid, Sulphuric acid, Atomic absorption spectrophotometer.

3.2 METHODS

3.2.1 SAMPLE COLLECTION AND PROCESSING

The matured avocado fruits (*P. americana*) were bought from a local market here in Benin located at Oredo Local Government Area of Edo State, Nigeria. It was identified in the Plant Biology and Biotechnology Department of the Faculty of Life Sciences, University of Benin, Nigeria with the hyberium number UBH-P408. The pods were cut in half with the aid of a sharp knife and the seeds were removed, rinsed in water and air-dried. The dried seeds were cut into

smaller pieces and further air-dried for at least 2 weeks and after which they were pulverised and stored till needed for later use at room temperature

3.2.2 ANTI-NUTRITIVE COMPONENTS

3.2.2a DETERMINATION OF PHYTATE ACID

The procedure of Lucas and Markakas (1975) was used to determine the phytate content of the seeds. 2 g of the sample was weighed (w/v) into a 250 ml conical flask. Roughly 100 ml of 2% Conc HCl was utilized to drench the example for 3 hours and then filtered with a Whatman No 1 filter paper. Around 25 ml aliquot of the filtrate was put in a different 250 ml funnel-shaped jar and 5 ml of 0.3% ammonium thiocyanate arrangement was included pointer. Roughly 53.5 ml of reined water was included and this was then titrated with standard iron III chloride arrangement which contains 0.00195 g of iron /milliliter until an earthly yellow shading continued for 5 minutes. Phytic corrosive was determined as in Eq.1

Phytic acid (%) = Titre value * 0.00195*1.19*100 …… Eq. 1

3.2.2b OXALATE DETERMINATION

The altered technique (Agbaire, 2011) was utilized to determine the oxalate content of the plant. Roughly 1 g of the seed oil (w/v) was weighed into a cone-shaped jar, and 75 ml of 3 m H_2so_4 was included and mixed with an attractive stirrer for 60 minutes. This was sifted and 25 ml aliquot of the filtrate was gathered and warmed to 80-90^{O}C. This filtrate was kept above 70^{o}C consistently. The hot aliquot was titrated against 0.05 M of $KMnO_4$ until an amazingly black-out

pale pink shading continued for 15-30 sec. The oxalate content was determined by taking 1 ml of 0.05 M of $KMnO_4$ as proportional to 2.2 mg oxalate.

3.2.3 DIGESTION AND DETERMINATION OF HEAVY METALS IN AVOCADO SEEDS

3.3 NITRIC – PERCHLORIC ACID DIGESTION

PROCEDURE

The concentrations of different heavy metals in the fruits were determined using the wet acid digestion method. Briefly, 0.5 g of the homogenized fruit sample was placed in a cleaned digestion tube, to which, 10 mL of mixed nitric acid and perchloric acid were further added, followed by stirring with glass rods. The digestion tube was then placed on the heater and heated inside a fume cupboard until its contents became colorless. The digested sample was cooled and filtered into a 100 mL volumetric flask, and about 10 ml of deionized water was added up to the mark on the flask. The solution was then aliquoted into metal-free sample bottles in triplicate and labeled properly for subsequent metal analysis. A reagent blank was prepared without the sample in the same way as the sample. The concentrations of different heavy metals (iron, copper, cobalt, zinc, lead, nickel, manganese, and chromium) in the digested solutions of each sample were determined using a graphite furnace atomic absorption spectrophotometer (GFAAS)

3.2.4 Working Principle Of Atomic Absorption Spectrophotometry (AAS)

Atomic absorption spectrophotometry (AAS) is an analytical technique used to measure a wide range of elements in materials such as metals, pottery, whole blood, serum, plants, glass e.t.c.

Although it is a destructive technique (unlike ED-XRF), the sample size needed is very small (typically about 10 mg - i.e. 100 of a gram) and its removal causes little damage. The sample is accurately weighed and then dissolved, often using strong acids. The resulting solution is sprayed into the flame of the instrument and atomized (see schematic diagram). Light of a suitable wavelength for a particular element is shone through the flame, and some of this light is absorbed by the atoms of the sample. The amount of light absorbed is proportional to the concentration of the element in the solution, and hence in the original object. Measurements are made separately for each element of interest in turn to achieve a complete analysis of an object, and thus the technique is relatively slow to use. However, it is very sensitive and it can measure trace elements down to the part per million level, as well as being able to measure elements present in minor and major amounts.

CHAPTER FOUR

4.0 RESULT

Table 4.1: The antinutritive components of *P. americana* seed powder

Parameters	Concentration
Phytate(mg/g)	1.07
Oxalate(mg/g)	6.16

Values indicated are single values (n=1)

The result shows that the avocado seeds contain a low amount of anti-nutrient properties, where oxalate contents are more than the phytate content below.

Table 4.2: Some minerals composition of *P. Americana* seeds powder

Parameters	Composition (mg/kg)
Iron(Fe)	2.775 ± 0.105
Zinc(Zn)	3.415 ± 0.145
Lead (Pb)	0.035 ± 0.005
Nickel (Ni)	0.115 ± 0.005
Copper (Cu)	0.265 ± 0.015
Cobalt (Co)	0.14 ± 0.01

Chromium (Cr)	0.08 ± 0

Values represented as MEAN ±SEM, n=2

The result shows that avocado seeds contain a significant amount of Heavy metals. Where Zinc (3.415 mg/g ± 0.145) and Iron (2.775 mg/g ± 0.105) have the highest amount. Lead (0.035 mg/g ± 0.005) and Chromium (0.08 ± 0) have the lowest amount of composition in the seeds.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.0 DISCUSSION

The avocado pear seed, which is typically thrown away after eating the avocado fruit flesh, may contain vital minerals and nutrients that are beneficial to human health. The anti-nutritional qualities and heavy metal content of avocado seeds were thus discovered by this investigation.

According to Degnon *et al.,* (2013), the oxalate content of avocado pear seed was significantly higher than the oxalate level of soursop fruit. Oxalate binds to calcium to create calcium-oxalate crystals, which are deposited as urinary calcium (stones) and are linked to renal tubule obstruction (Blood and Radostits, 1989). These anti-nutrients would be reduced by proper food

processing. Avocado seeds were found to have a low concentration of phytic acid when compared to the African wild mango (Oboh and Ekperigin, 2003). This finding was in collaboration with this study where the phytic acid content was found to be low.

The antinutritional nature of phytic acid lies in its ability to chelate divalent minerals such as iron, calcium, copper and zinc, rendering them unavailable to the body biologically. The phytic acid concentration of avocado seeds was significantly decreased during processing (soaking and boiling) (Justina *et al.,*2016). Adegoke et al, (2012) reported higher values for phytic acid and oxalate in avocado seeds. However, boiling for 25 minutes and soaking for 24 hours effectively reduced the anti-nutritional factors without hurting the nutritional quality. When compared to avocado seeds, the anti-nutritional components of *Terminalia catappa* (almond) seeds have higher values (Akpabio, 2012). The lethal levels reported by Inuwa et al, (2011) were lower than the anti-nutrient parameters found in the present study. Given that the majority of the values are below the lethal doses of these phytochemicals, it may suggest that the quantities of oxalate and phytate in avocado seeds are deemed modest and would not be harmful for ingestion. It has significant nutritional levels that can support testing and its use in feed formulation.

Copper (Cu) is an essential trace element that, within reasonable limits, is necessary for good health. In the same way as its high uptake in fruits can be detrimental to human health inducing liver damage and other gastric-related problems (Gaetke and Choul, 2003). Its low uptake in human consumption can result in a variety of symptoms like growth retardation, skin conditions, gastrointestinal disorders, etc. The value of Copper(Cu) as seen in this study was found to be lower as compared to work done by Radwan and Salama, (2006) as seen in grapefruits.

Many essential enzymes contain zinc (Zn), which is a significant component. Any abrupt oral ingestion might cause symptoms like vomiting, tachycardia, nausea, vascular shock, and

pancreatic dysfunction. The concentrations of Zn from this study were found to be low compared to its nutritional standard. All of the tested two samples results from this investigation were found to be significantly lower than the maximum permissible.

Iron (Fe) is a necessary component of human beings and is crucial for the body's production of hemoglobin, oxygen, and electron transport. The iron concentrations for avocado pear in this study were found to be significantly higher than the iron concentration for raspberries, which was reported by Plessi et al. (2007)

The findings of this study revealed that lead (Pb) concentrations in avocado seeds are lower compared to those in pawpaw as reported by Kalagbor and Diri (2014). The buildup of lead (Pb) in the brain causes plumbism. It causes lower IQs, short attention spans, hyperactivity, and mental decline in children. Adults have suffered memory loss and joint deterioration.

Nickel (Ni), is an element that occurs in the environment only at extremely low levels and is essential in small doses, but it can be dangerous when the maximum tolerated amount is exceeded. This can cause various kinds of cancer on various sites of the body. Its concentration in the sample tested was significantly lower which is comparable to the result obtained by Orisakwe et al, (2012) for pineapple.

Avocado seeds were found to have cobalt contents that were lower than the maximum permissible limit. Sobukola et al. (2010) showed even lower levels for oranges (0.027 mg/kg), and pineapple (0.022 mg/kg). Co has similar characteristics to Fe and Ni. Excessive exposure to Co can have negative effects on the kidneys, thyroid, liver, and heart.

The maximum permissible limit for chromium (Cr) in food is 0.05 mg/kg, whereas avocado seeds have a chromium value of 0.08 mg/kg. Agenda et al. (2015) observed even lower levels for cashew (0.025 mg/kg). Depending on its oxidation state chromium may either be advantageous or dangerous. Through a variety of anthropogenic and natural processes, chromium is introduced to the environmental media (soil, water, and air) in the form of chromium (III) and chromium (VI). Chromium (III) is frequently taken up by plants. It is important in the breakdown of fats and carbohydrates and may potentially have antioxidant properties. It promotes the production of cholesterol and fatty acids. They are crucial for the operation of the brain and other bodily systems. Additionally, chromium supports insulin function and glucose metabolism.

Excessive exposure to Chromium causes gastrointestinal distress, headaches, skin rashes, insomnia, mood swings, and potentially liver or kidney damage (David *et al.,* 2021).

5.1 CONCLUSION

Given that the majority of the values are below the lethal doses of these phytochemicals, it can be concluded that the levels of oxalate and phytate in avocado seeds are deemed modest and would not be harmful for animal ingestion. It has significant nutritional levels that can support testing and use in feed formulation about mineral contents.

REFERENCES

Adda, B. (2020). Everything you need to know avocado. Extracted from https://www.medicalnewstoday.com/articles/318620

Adegoke, G.O., Akinbile, J.T., Olapade, A.A. and Ashaye, O.A. (2012). The effect of processing methods on the nutritional profile of avocado (Persea americana Mill) seeds. *International Journal of Recent Research and Applied Studies* **11(2):** 186-194.

Akpabio, U.D. (2012). Evaluation of proximate composition, min-eral element and anti- nutrient in almond (*Terminalia catappa*) seeds. *Advance Applied Science Research* **3(4):** 2247-2252.

Araújo, R.G., Rodríguez-Jasso, R.M., Ruiz, H.A, Govea-Salas, M., Rosas-Flores, W. and Aguilar-González, M.A. (2020). Hydrothermal – microwave processing for starch extraction from mexican avocado seeds: Operational conditions and characterization. *Processes* **8(7):**1-17.

Armstrong, W.P (2000). "Fruits of The Rose, Olive, Avocado & Mahagany Families – Laurel Family: Lauraceae. Extract from: http://waynesword.palomar.edu/ecoph17.html

Asha, M.R., Hithamani, G., Rashmi, R., Basavaraj, K.H., Rao, J.K.S., and Rao, T.S.S. (2009). Nutritional and antinutritional composition of processed Avocado (Persea Americana Mill) seeds. *Indian Journal of Psychiatry* **51(2)**: 141-149.

Ashman, T.L., Knight, T.M., Streets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mazer, S.J. and Mitchell, R.J. (2004). Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. **Ecology 85(9):** 2408-2421.

Atli, A. (2022). Phytic Acid 101: Everything You Need to Know. Extracted from https://www.healthline.com/nutrition/phytic-acid-101

Blood, D.C. and Radostits, O.M. (1989). Veterinary Medicine: A Textbook of the Diseases Of Cattle, Sheep, Pigs, Goats and Horses. pp. 589-630.

Carolyn, T. (2018). Peak Season for Avocados. Extracted from https://www.weaverstreetmarket.coop/peak-season-for avocados/#:~:text=Avocados%20are%20available%20year%20round,time%20of%20year%20for%20flavor.

Cheryan, M. (1980) Phytic acid interactions in food systems. *CRC Critical Reviews in Food Science and Nutrition* **13**: 297- 335.

Corinne, K. (2015). All About Pinkerton Avocados. Extracted from

https://www.mexicado.com/blog/all-about-pinkerton-avocados/

Cosgrove, D.J (1966). The chemistry and biochemistry of inositol polyphosphates. *Reviews of Pure and Applied Chemistry 16*:209-224.

Cosgrove, D.J. (1980). Inositol Phosphates. Their Chemistry, Biochemistry and Physiology. *Elsevier Scientific Publishing Company* p. 81.

Dabas, D., Shegog, R.M., Ziegler, G.R. and Lambert, J.D. (2013) Avocado (*Persea americana*) seed as a source of bioactive phytochemicals. *Current Pharmaceutical Design* **19(34)**: 6133-6140.

David, H., Myers, J. and Stokes, C. (2008). Heavy Metals in North Carolina Soil Occurrence and Significance. *Department of Agriculture and Consumers Service Publication. North Carolina digital collections* p. 1.

David, B., Olufunso, A., Adebayo, A. and Oluwole, A. (2021). Heavy Metal Concentration and Health Risk Assessment of Selected Fruits Sold in Jos Metropolis. *Advances in Chemical Research* **3(2):**210.

Deepti, D., Rachel, M., Gregory, R.Z. and Joshua. D.L. (2013). Avocado (*Persea americana*) seed as a source of bioactive phytochemicals. *Current Pharmaceutical Design* **19(34)**:6133-40.

Degnon, M.R., Adjou, E.S. and Noudogbessi, J.P. (2013). Investigation on nutritional potential of soursop (Annonamuricata L.) from Benin for its use as food supplement against protein-energy deficiency. *International Journal of Biosciences* **3(6):** 135-144.

Diana, L., Hsin-I, W., Wan-Jen, W. and Ray-Yu, Y. (2018). Anti-nutrient components and their concentrations in edible parts in vegetable families. *CAB Reviews* **13(15):**1-30.

Dorcas, O.J, Joseph, N.E. and Sunday, J.S. (2016). Determination of the contamination levels of cadmium, lead, arsenic and mercury in the vegetables from old mining site of Jos Plateau, Nigeria. *International Journal of Interdisciplinary Research and Innovations* **4**:40-44.

Eduardo, P.C., Moisés M.V., José Miguel, F.F. and Socorro, V.R. (2013). Acute Toxicity and Genotoxic Activity of Avocado Seed Extract (*Persea americana* Mill., c.v. Hass). *The Scientific World Journal* **13(10)**:245828.

Egbenda, O., Thullah, P. and Kamara, F. (2015). A physico-chemical analysis of soil and selected fruits in one rehabilitated mined out site in the Sierra Rutile environs for the presence of heavy metals: Lead, Copper, Zinc, Chromium and Arsenic. *Academic journal* **9(2):**27-32.

Fabio, D. (2016). Avocado Tree. Extracted from

https://www.learnaboutnature.com/plants/trees/avocado-tree/

Fanny, L., Marie-Anne, L.V., Elisabeth, C. and Christian, R. (2005). Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *The Journal of Agricultural and Food Chemistry* **53(1):**98-102.

Fasasi, F. (2018). 5 Health Benefits Of Avocado (Pear) You Should Know. Extracted from https://ijebuloaded.com/5-health-benefits-avocadopear-know/

Figueroa, J.G., Borrás-Linares, J.I., Lozano-Sánchez, Q.R. and Segura-Carretero, A. (2018). Optimization of drying process and pressurized liquid extraction for recovery of bioactive compounds from avocado peel by-product. E*lectrophoresis* **39(15):**1908-1916.

Gustafsson, E.L. and Sandberg, A.S. (1995). Phytate Reduction in Brown Beans (*Phaseolus vulgaris L.*). *Journal of Food Science* **60(1):**149-152.

Gaetke, L.M. and Chow, C.K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology* **189**:147–163.

Heaney, R.P. and Weaver, C.M. (1989). Oxalate: effect on calcium absorbability. *The American Journal of Clinical Nutrition* **50(4):** 830–832,

Inuwa, H.M., Aina, V.O., Gabi, B., Aimola, I. and Toyin, A. (2011). Comparative determination of antinutritional factors in groundnut oil and palm oil. *Advance Journal Food Science Technology* **3(4):** 275-279.

Josie, S. (2016). Not all avocados are created equal. Extracted from

https://i.stuff.co.nz/life-style/food-wine/84454679/not-all-avocados-are-created-equal

Justina, Y.T., Olukemi, A.O., Ajayi, O.O. & Adegoke, G.O. (2016). Nutritional and antinutritional compositions of processed Avocado (*Persea americana* Mill) seeds. *Pelagia Research Library Asian Journal of Plant Science and Research* **6(2):** 6–12.

Kalagbor, I. and Diri, E. (2014). Evaluation of heavy metals in orange, pineapple, avocado pear and pawpaw from a farm in Kaani, Bori, Rivers State Nigeria. *International Research Journal of Public and Environmental Health* **1(4):**87-94.

Kawagishi, H., Fukumoto, Y., Hatakeyama, M., He, P., Arimoto, H. and Matsuzawa, T. (2001). Liver injurysuppressing compounds from Avocado (*Persea americana*). *Journal Agricultural Food Chemistry* **49(25):**2215-2221

Lara-Marquez, M., Baez-Magana, M., Raymundo-Ramos, C., Spagnuolo, P.A., Macias-Rodriguez, L. and Salgado-Garciglia, R. (2020). Lipid-rich extract from Mexican avocado (Persea americana var. drymifolia) induces apoptosis and modulates the inflammatory response in Caco-2 human colon cancer cells. *Journal of Functional Foods* **64:**103658

Lydia, P.J., Sumi, R., Pooja, R.A., Rajasekaran, R., John, J.A. and Mehanathan, M. (2021). Diverse role of phytic acid in plants and approaches to develop low-phytate grains to enhance bioavailability of micronutrients. *Advanced Genetics.* **107:**89-120

Małgorzata, G. and Piotr, S. (2013). Comparative assessment of essential and heavy metals in fruits from different geographical origins. *Environmental Monitoring and Assessment* **185**:9139–9160.

Masashi, H. (2014). Benefits, risks and mechanisms of whole grains in health promotion. pp 181-199

Maxipharo. (2020) Hass Avocado farming in Nigeria Extract from https://agricincome.com/hass-avocado-farming-in-nigeria/

Melgar, B., Dias, M.I., Ciric, A., Sokovic, M., Garcia-Castello, E.M., Rodriguez- Lopez A.D. (2018).

Bioactive characterization of Persea americana Mill. by-products: A rich source of inherent antioxidants.

Industrial Crops and Products 111: 212-218

Miller, A.W., Dale, C. and Dearing, M.D. (2017). The induction of oxalate metabolism in vivo is more effective with functional microbial communities than with functional microbial species. *mSystems* **2(5):**8-17.

Mónica, L.M., Marisol, B.M., Cristina, R.P., Rafael, S.G., Alejandra, O.Z. and Joel, E.L. (2020). Lipid-rich extract from Mexican avocado (Persea americana var. drymifolia) induces apoptosis and modulates the inflammatory response in Caco-2 human colon cancer cells. *Journal of Functional Foods* ***64***:103658.

Morton, J.F. (1987). Fruits of warm climates. Economic Botany. **4**: 91-102.

Nabrzyski, M. (2007). Functional role of some minerals in foods. In P. Szefer & J. O. Nriagu (Eds.), Mineral components in foods. pp. 363–388.

Nayak, B. S., Raju, S. S. and Chalapathi-Rao, A. V. (2008). Wound healing activity of Persea americana (avocado) fruit: a preclinical study on rats. *Journal Wound Care* **17(3):** 123-126.

Nwaogu, L.A., Alisi, C.S. and Ojiako, O.A. (2008). *Bio-Research* **6(1):** 320-322.

Odugbemi, T. (2006). Medicinal plants as antimicrobials. In: Outline and pictures of Medicinal plants from Nigeria. University of Lagos press pp.53-64.

Onakpa, M.M., Njan, A.A. and Kalu, O.C. (2018). A review of heavy metal contamination of food crops in Nigeria. *Annals of Global Health* **84**: 488-494.

Orhevba, B.A. and Jinadu, A.O. (2011) Determination of Physico-Chemical Properties and Nutritional Contents of Avocado Pear (*Persea americana M*.). *Academic Research International* **1(3):** 369-374.

Orisakwe, O.E, Nduka, J.K, Amadi, C N., Dike, D.O. and Bede, O. (2012). Heavy metals risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern Nigeria. *Chemical Centre Journal* **6:** 77–83.

Parker, O. (2022). What Is a Hass Avocado? Extracted from

https://www.delightedcooking.com/what-is-a-hass-avocado.html

Plessi, M., Bertelli, D. and Albasini, A. (2007). Distribution of metals and phenolic compounds as a criterion to evaluate variety of berries and related jams. *Food Chemistry* **100:** 419–427.

Radwan, M. and Salama, A. K. (2006). Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Food and Chemical Toxicology* **44:** 1273–1278.

Ramos, M.R., Jerz, G., Villanueva, S., López-Dellamary, F., Waibel, R.P. and Winterhalter P. (2004). Two glucosylatedabscisic acid derivates from avocado seeds; (*Persea americana* Mill. *Lauraceae cv.* Hass). *Phytochemistry* **65(7):** 955-962.

Ran, P., Michael, L., Helen, M., O'Connor, Xujian, Li, Eric B. and John, C. L. (2012). A controlled metabolic diet reduces calcium oxalate supersaturation but not oxalate excretion after bariatric surgery. *Urology* **80(2):** 250–254.

Sandström, B., Almgren, A., Kivistö, B. and Cederblad, A. (1989). Effect of protein level and protein source on zinc absorption in humans. *Journal of Nutrition* **119(1)**:48-53.

Sandrine, P., Luc, D., Anne-Claire, V., Carla, E., Emmanuelle, K., Sandrine, B., Paule, L., Serge, H. and Pilar, G. (2008). Relationship between iron status and dietary fruit and vegetables based on their vitamin C and fiber content. *The American Journal of Clinical Nutrition* **87(5):**1298-305.

Sobukola, O.P., Adeniran, O.M., Odedairo, A.A. and Kajihausa, O.E. (2010). Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. Africa. *Journal Food Science* **4(2):** 389-393.

Soledad C.P.T., Paola H.C., Enrique C., Israel R.L.I., Guadalupe N.M. and Raúl A.S. (2021). Avocado seeds (Persea americana cv. Criollo sp.): Lipophilic compounds profile and biological activities. *Saudi Journal of Biological* **28(6):** 3384-3390.

Steve, A. (2016). How to Plant, Grow, and Harvest Avocados. Extract from https://harvesttotable.com/how-to-plant-grow-and-harvest-avocados/

Swisher, H.E. (1988). Avocado Oil, from Food Use to Skin Care. *Journal of the American Oil Chemists Society* **65(11):** 1704-1713.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J. (2012). Heavy metal toxicity and the environment. *Molecular, Clinical and Environmental Toxicology* **101**: 133-164.

Thomas, L.D. (2018). Cross- vs. self-pollination in 'Hass' avocados growing in coastal and inland orchards of Southern California. *Scientia Horticulturae* **246:** 307-316

Tina, M. (2019). Eating the Avocado Seed Isn't Actually Safe — Here's How to Get Those Nutrients Instead Extracted from https://www.livestrong.com/article/31737-eat-avocado-seeds-nutrition/

Tremocoldi, M.A, Rosalen, P.L., Franchin, M., Daiuto, R., Augusto, J. and Massarioli, P. (2018). Exploration of avocado by-products as natural sources of bioactive compounds. *PLoS ONE* pp. 1-12

Vanya, S. (2020) Amazing Benefits Of Avocado Seeds. Extract from https://www.organicfacts.net/avocado-seeds.html

Villarreal-Lara, R., Rodríguez-Sánchez, D.G., Díaz De La Garza, R.I., García-Cruz, M.I., Castillo, A. and Pacheco, A. (2019). Purified avocado seed acetogenins: Antimicrobial spectrum and complete inhibition of Listeria monocytogenes in a refrigerated food matrix. *Journal of Food* **17 (1):** 228-239.

Weston, P. and Deanna, M. (2020). Is There Such a Thing as "Anti-Nutrients"? A Narrative Review of Perceived Problematic Plant Compounds. *Nutrients* **12(10):**2929.

Yianna, Y.Z., Regine, S., Ken, N. and Said, A. (2020). Revisiting phytate-element interactions: implications for iron, zinc and calcium bioavailability, with emphasis on legumes. *Critical Reviews in Food Science and Nutrition* **62(6)**:1696-1712.