EFFECT OF PROCESSING ON CYANIDE CONTENT AND FUNCTIONAL PROPERTIES OF CASSAVA SEED PROTEIN CONCENTRATE

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,
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BIOCHEMISTRY

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DECLARATION

I hereby declare that this thesis titled: **"Effect of Processing on Cyanide Content and Functional Properties of Cassava Seed Protein Concentrate"**, is a collection of my original research work and it has not been presented for any other qualification anywhere. Information from other sources (published or unpublished) has been duly acknowledged.

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CERTIFICATION

This thesis titled: ***"Effect of Processing on Cyanide Content and Functional Properties of Cassava Seed Protein Concentrate"*** *by GANA, Blessing Kakawusa*

(MTech/SLS/2018/8485), meets the regulations governing the award of the degree of Master of Technology in Biochemistry, Federal University of Technology, Minna, and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty and my parents Dr. and Mrs Peter J. Gana.

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ABSTRACT

Cassava seed is rich in proteins. However, it less exploited partly due to the high cyanide level of the entire cassava plant and mostly due to the fact that cassava seed is not well known. The effects of processing on cyanide content and functional properties of cassava seed protein concentrate were determined. Determination of proximate composition, antinutrient composition and the functional properties were carried out using standard methods. Effects of processing methods such as boiling, roasting and fermentation on the cyanide content of cassava seed protein concentrate were determined. The cassava seed was defatted by solvent extraction. The protein concentrate was prepared from the flour samples using isoelectric precipitation extraction method. The amino acids profile was determined using automated amino acid analyzer. The protein recovery was 84.31%. The proximate analysis showed that carbohydrate, ash and crude protein were significantly ($p < 0.05$) lower in the raw cassava seed flour compared to the defatted cassava seeds. The functional properties of the cassava seed concentrate showed that swelling capacity was the highest with 277.18 ± 16.97% and bulk density was the lowest with 0.75 ± 0.78 g/ml. a total of 18 amino acids were obtained from cassava seed protein concentrate, the total amino acid content was 79.88 ± 7.99 g/100g, with 48.75 ± 3.49 g/100g of non-essential amino acids and 29.76 ± 2.56 g/100g of essential amino acids, and lysine was the limiting amino acid (amino acid score = 0.88). The anti-nutritional contents (flavonoids, alkaloids, tannins and cyanide were significantly ($p < 0.05$) lower in the defatted cassava seed flour compared to the raw cassava seed flour. However, the anti-nutritional contents determined were significantly ($p < 0.05$) lower in protein concentrate compared to raw samples. Though the three processing methods (fermentation, roasting and boiling) significantly ($p < 0.05$) lowered cyanide in cassava seed protein concentrate, boiling was more effective. Extensive boiling of cassava seed protein concentrate for 26 hours reduced the cyanide levels to negligible amount of 0.91 ± 0.15 mg/100g. The findings have shown that adequate processing can reduce the cyanide content of cassava seed protein concentrate to innocuous level and allow for its full potential usage as food.

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LIST OF ABBREVIATIONS

LD: Lethal Dose

TAN: Tropical Ataxic Neuropathy

FAO: Food and Agriculture Organization

HCN: Hydrogen Cyanide

VPD: Vapor Pressure Deficit

PPD: Postharvest Physiological Deterioration

CNG: Cyanoglucosides

LDL: Low Density Lipoprotein

HDL: High Density Lipoprotein

GPR: Guaranteed Pure Reagent

WAC: Water Absosrption Capacity

RNA: Ribonucleic Acid

DNA: Deoxyribonucleic Acid

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The third-largest source of carbohydrates for food in tropical areas, after rice and maize, is the perennial root crop known as cassava (*Manihot esculenta*). As a food or industrial crop, it is one of the most popular tuber crops growing throughout Africa, Asia, Central and South America, and also, in other parts of the world (Ferraro *et al*., 2016). It is a

significant staple crop in many Sub-Saharan African nations and developing nations, providing nearly 500 million people with a basic diet. It is the most valuable crop in terms of production and the second-most valuable crop in terms of consumption (Nanbol and Namo, 2019).

Plant breeders frequently use true cassava seeds to develop improved species, and vegetative propagation occurs either asexually from seedlings or asexually from cuttings of stems, meristems, leaf buds, and roots (Delaquis *et al*., 2018). Production appears to have the potential to reduce or eliminate hunger.

Many varieties of the crop bear a lot of fruit, the fruits are dehiscent, with three seeds embedded in a capsule. The seeds resemble castor seeds (*Ricinus communis*) in size and shape. Apart from cultivation, certain regions of Gabon consume cassava seeds, which are said to "taste like hazel nuts". Evidently, this is the only area cassava seeds are consumed (Nartey and Møller, 1976).

In general, seeds are good sources of healthy fats, minerals (like potassium, magnesium, plant iron, zinc and calcium), some vitamins (B1, B2, and E), and proteins. Additionally, antioxidants found in oily seeds stop fats from quickly going rancid (Kang *et al*, 2017; Adeleke and Babalola, 2020). Given their distinctive nutrient profiles, seeds have many health benefits, these include the ability to regulate weight, lower risk of diabetes and heart disease, and its anti-ulcer, immuno-modulatory, and anticancer properties (Singh *et al*., 2019).

Cassava seed kernels were found to contain 47 % lipids and 34 % proteins (Nartey *et al*., 1973; Nartey *et al*., 1974), implying a high protein content. Cassava seeds, which are highly nutritious, are high in oil and fatty acids (Alves *et al*., 2014).

Consuming foods containing cyanogenic glycosides, such as almonds, cassava, etc., can expose one to cyanide (HCN or CN^-, expressed as CN) (Tran *et al*., 2020). The Bglucosidase enzyme, linamarase, or a combination of high heat and strong acids can break down the relatively stable cyanogenic glucosides (cyanoglucosides) to liberate cyanohydrin and glucose. Due to its instability above pH 5.5, cyanohydrin breaks down into acetone and hydrogen cyanide. Although it is generally known that the lethal dose of HCN for humans is between 0.5 and 3.5 mg/kg body weight, there is still debate regarding the deadly concentrations of other cyanogenic chemicals present in cassava (Hill and Roberts, 2020).

For cassava to be converted into a safe food or feed, its harmful glucosides must be removed by adequate processing (Ndam *et al*., 2019). In general, food processing is the process of converting raw materials into intermediate foodstuffs or edible products using scientific knowledge and technology. Fresh foods are transformed into food products by food processing (Misra *et al*., 2017). Bulky, perishable, and possibly inedible food components are converted into more beneficial, concentrated, shelf-stable, and delicious foods via a variety of techniques. This may involve one or more of the following operations: washing, chopping, pasteurization, freezing, fermenting, packaging, cooking, and a lot more. By processing cassava effectively, Cyanide-yielding substances are normally reduced to negligible levels (Quinn *et al*., 2022).

Protein concentrates are dietary supplements for humans and animals that are extracted or produced from plant or animal matter and have a high protein concentration. They are made from defatted protein-containing flour after non-protein dietary components like antinutritional factors, carbohydrates, some low molecular weight nitrogenous compounds and soluble minerals are eliminated from food (Rahate *et al*., 2021; Neji *et al*., 2022). Proteins in this form are required ingredients in many food processes because they improve nutritional quality without affecting other properties significantly, and in

some cases, the addition of protein concentrate appears to extend final product shelf life (Sharifi-Rad *et al*., 2020).

1.2 Statement of Research Problem

The need for protein among the rapidly expanding populations in many developing nations is greater than the supply of animal protein can provide. As a result, there is a corresponding increase in the cost of various plant-based protein sources. Since there is a continuous increase in the cost of animal protein sources, there is a greater research interest on food characteristics and alternate sources of plant-based proteins from locally accessible crops at a lower cost of production, particularly from underutilized or relatively ignored high protein oilseeds and legumes (Cheng *et al*., 2017).

Cassava seed could be a valuable economic and nutritional source of proteins in addition to fats (Nartey *et al*.,1973; Nartey *et al*., 1974). Furthermore, cassava seeds are potential sources of essential amino acids (Nartey *et al*., 1976); however, due to its high cyanide content, it has not been widely incorporated into the food system as an alternative protein source, and thus it is wasted. In fact, there is high level of cyanide in all the parts of cassava i.e. the cassava plant's leaves, stems, roots have high cyanide levels, primarily linamarin and lotaustralin. It is worth noting that, linamarin is the most abundant cyanogen, it produces HCN via an intermediate compound called cyanohydrin (De Jesus *et al*., 2018; Nyirenda, 2020).

Cyanide contains cyanogenic glycosides, primarily lotaustralin and linamarin, 2-Dglucopyranosyloxy-2-methylbutyronitrile and 2-D-glucopyranosyloxy-2-methylpropane nitrile, respectively. The cyanogens in the human gut hydrolyze to generate toxic hydrocyanic acid, which is linked to a number of health issues including epilepsy, thyroid goiter, Konzo disease, tropical ataxic neuropathy (TAN), cretinism

cases, particularly, in children, it causes growth retardation and abnormalities in both emotions and behaviour (Kariuki *et al*., 2017; Mushumbusi *et al*., 2020).

Despite the development of numerous processing techniques and their application in removing antinutrients from many seeds, their role in reducing cyanide in cassava seeds remains unknown. This contributes to the seed's global underutilization, particularly as a potential protein source.

1.3 Justification of Study

The average protein intake in developing nations is lower than required, owing in part to rapid population growth. Plant proteins can meet this requirement, and as a result, they play important roles in human nutrition. An increasing number of food systems around the world are using plant protein products as ingredients, and their distinct flavour in food encourages their use as additives.

Numerous research on the protein function of major and minor oilseeds, including peanut, soybean, sunflower, rapeseed, almond, ground nut and winged bean have shown the significance of oilseeds as sources of lipids and plant proteins (Adenekan *et al*., 2017). They can therefore be useful in augmenting protein intake which can be achieved by either diet supplementation, development of new products or fortification of different starchy staples.

Cassava seed has excellent nutritional values as an oil seed, including a high protein content with essential amino acids (Nartey *et al*.,1973; Nartey *et al*., 1974) and it can become a rich protein source. Therefore, cassava seed protein concentrate supplementation will decrease reliance on commercially manufactured foods and improve the use of this underutilized local staple (cassava) seeds. Furthermore, cassava seed is

cost effective, easily transported because of its size, it is readily available because they are not eaten and easily accessible because cassava plant can be easily grown. Application of suitable processing methods will augment its utilization.

The high level of cyanide in cassava seeds can be decreased to tolerable levels for possible incorporation in human diets by using suitable processing methods that have already been applied to other cyanide-yielding substances (Ajibola and Olapade, 2016; Muleta and Mohammed, 2016; Akinsanmi *et al*., 2020). Traditionally, cyanide in cyanide-yielding substances is processed using a variety of techniques (such as leaching/ soaking, fermentation, boiling/cooking, and drying) that vary greatly from place to region (Gulukun *et al*., 2020). In general, various processing techniques for reducing cyanide in cyanide-yielding substances for industrial purposes have been developed (Ndubuisi and Chidiebere, 2018; Adepoju *et al*., 2019), and integrating their application to cassava seeds will be invaluable.

1.4 Aim of Study

The aim of the study was to determine the effects of processing on cyanide content and functional properties of cassava seed protein concentrate.

1.5 Objectives of the Study:

The objectives of this study are to determine:

i. The proximate composition of whole and defatted cassava seed flour

ii. The anti-nutrient composition of whole, defatted and protein concentrate of cassava seed iii. The functional properties of cassava seed protein concentrate iv. The amino acid composition of cassava seed protein concentrate

v. The effects of processing on cyanide content of cassava seed protein concentrate

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of Cassava

The perennial woody shrub known as cassava (*Manihot esculenta* Crantz) is a member of the Malpighiales order and of the Euphorbiaceae family (spurge family). It is also known as yuca, kamoteng kahoy (in Spanish), balinghoy (in Filipino), manioc, mandioc, mogo (in Swahili), and tapioca (Panghal *et al*., 2019). It is the genus's only domesticated species, hailing from the western Amazonian Rim, and was first grown by Amerindian Indians. It is a perennial with a brief lifespan that reproduces vegetatively. Its starch-filled tuberous roots grow close to the soil's surface. After the advent of Europeans in the Americas, Portuguese traders transported crop varieties to Africa and Asia, where they flourished in analogous tropical regions (Scott, 2021).

The cassava shrub has leaves that are finely divided into 3–7 lobes and can grow to a height of 2.75 meters. For a C3 plant, cassava has relatively high leaf photosynthesis when soil moisture is easily accessible and air humidity is high (Vongcharoen *et al*., 2018). During extended times of water scarcity, a leaf's conductance retains a high inner water balance and even some development by a powerful feed-forward regulatory response to vapor pressure deficit (VPD). When VPD surpasses 1-2 kPa, even while solar radiation, availability of soil water and temperature are favourable for development, the response to VPD lowers photosynthesis. The VPD response reduces total biomass production while

making cassava drought resistant. In tropical environments, the increased harvest index and continuous growth make up for the limited production of total biomass (Widuri *et al*., 2020).

Cassava cultivation and consumption increased in the middle of the nineteenth century and spread far throughout Africa (Otekunrin and Sawicka, 2019). Cassava production is thought to have started in Mexico and Guatemala more than 4,000 years ago, traveling from the north east region of Paraguay/ Brazil. It initially grown in the Congo Basin and the Gulf of Guinea and it is believed that Portuguese traders transported it to the western part of Africa around 1588. Later, the cultivation reached Madagascar and other parts of eastern Africa (De Bruyn *et al*., 2016).

2.2 Distribution of Cassava

Cassava is a very important root crops grown worldwide in both the tropical and in the subtropical regions. It is a major food crop for almost one billion people in 105 countries and is farmed between latitudes 300N and 300S in tropical Africa, the Americas, and Asia (Li *et al*.,2017). In addition, cassava is a staple food crop for nearly one billion people in 105 countries (Latif and Müller, 2015). The crop currently occupies 26.3 Mha worldwide.

Cassava is a drought-resistant crop that may be cultivated on marginal soils and in areas where rainfall patterns are unpredictable, resulting in the failure of many other crops. Contrary to other crops, cassava can be planted in areas with poor soil fertility and issues like exchangeable base deficiency, phosphorus fixation, soil erosion and excess aluminium concentration (Polthanee *et al*., 2016; Ezui *et al*., 2016), this allows for healthier soils to be available for crops that will provide higher profits. Cassava is mostly cultivated as a rain-fed crop and is rarely found in areas where average temperature each year is below 20 °C. It is typically grown in areas at which average rainfall annually is

above 1000 mm and in soils with good drainage to prevent waterlogging, which destroys the crop. Other than excessive rain and subsequent flooding, it is a strong plant highly suitable for adverse weather climates, and it is projected to become increasingly significant as regions appropriate for presently available crops decline owing to climate change (Vongcharoen *et al*., 2018). It can even be seen in locations with rainfall as low as 650–750 mm spread out across only 5 months. It thrives in a wide range of soil conditions and is particularly suited to low-fertility soils. When developing in such settings, canopies retain a high nutritional content in functional leaves and improve the quantity of biomass distributed to tubers, whereas crops inhibit top development.

2.2.1 World production of cassava

Cassava is a staple food for 800 million people in Asia, Africa, South America and the Caribbean. In the past decade, Africa and Asia have been primary cassava producers, in fact, in Africa and Asia, cassava is thought to be farmed on around 70 % of the estimated 13 million hectares of land (Ogbonna *et al*., 2021). In 2014, more than 54 % of the cassava consumed worldwide is grown in Africa, Nigeria produced 54.8 million tonnes (MT), the most of any country (Akomolafe *et al*., 2023). However, Nigeria's average output of 7.7 MT per hectare was quite low when compared to the mean yield of 23.4 MT and 22.2 MT per hectare generated in Indonesia and Thailand, the two other top producers of cassava in the world (Akomolafe *et al*., 2023). In 2017, it was estimated that 291 MT of cassava are produced worldwide, with Nigeria, Democratic Republic of the Congo, Thailand, and Indonesia placing first through fourth, respectively. Africa remained the world's greatest producer of cassava, with 177 MT produced there in 2017. Without a doubt, Nigeria continued to produce the most cassava in the world in 2017, producing over 59 MT (Nanbol and Namo, 2019). Nonetheless, in 2019, Brazil became the world’s second largest cassava producer after Nigeria (Otekunrin and Sawicka, 2019). All of

Nigeria's agro-ecological zones are used to grow cassava, although the rainforest and derived savannah regions are where it thrives. The most production occurs in the SouthSouth and North Central regions. After rice, wheat, maize, barley, and potato, cassava is the sixth-most significant crop in the world, and in the tropics, it is the most frequently farmed root crop (Saranraj *et al*., 2019). Almost all of Nigeria's states grow cassava, but only 11of them are known to be the country's major cassava producers. Taraba, Enugu, Anambra, Ogun, Kogi, Edo, Cross-River, Imo, Delta, Benue and Ondo are among the eleven states (Inoue *et al*., 2015; Wossen *et al*., 2017).

2.3 General Description of Cassava

2.3.1 Cassava plant parts

Cassava is the fourth-largest crop in the world and is grown in regions including Latin America, America, Asia, and Africa. It is a root crop with a lot of starch that offers a lot of calories in tropical areas. Additionally, it is employed in a variety of industrial applications as a raw (Ohimain, 2014). Stems, roots, and leaves are the three main plants of the cassava as well as seeds. The stems serve as transport organs, transferring manufactured food to various parts of plant for growth and development. They also offer planting products for a variety of allied crops (Edhirej *et al*., 2017).

2.3.1.1 Stems

The mature stem has an alternating pattern of internodes and nodes and is cylindric in shape. Protuberances the scars formed by the plant's first leaves are among the oldest part of stem’s nodule. The number of primary stems that can sprout from a stem cutting depends on the number of healthy buds present. Only one stem grows in some cultivars with significant apical dominance (Kinyua and Okwaro, 2021). There is sympodial branching in the cassava plant. The primary stem(s) branch out in successive directions

by di-, tri-, or tetrachotomously dividing into secondary branches. Reproductive branchings are the term used to describe these branchings that are brought on by flowering. A cultivar's stem morphological and agronomic traits are crucial for identifying it. These characteristics vary according to cultivar, cultural practice, and climatic conditions (Streck *et al*., 2014).

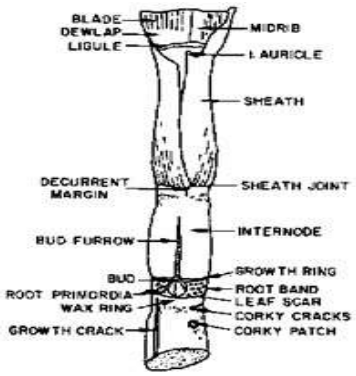


Figure 2.0: Cassava Stem

Source: Sulaiman *et al*. (2018)

2.3.1.2 Roots

The cassava plant has three types of roots: thick roots, fine white roots and tuberous roots. The thick roots aid in the anchoring the plant to the earth, carbohydrates are stored in the tuberous roots. In contrast, the fine white roots absorb nutrients and water (Spencer and Ezedinma, 2017).

The primary quality of cassava roots, which makes them the plant organ with the highest current economic worth, is their capacity to retain starch. Not all generated roots serve as storage organs, though. True seedlings grow a conventional primary tap root system, just like dicot species do. The germination seed's radical grows vertically downward and then, it will become a taproot where adventitious roots develop. The taproot and a few adventitious roots later develop into store roots. The roots of plants formed from stem

cuttings are adventitious, growing from the stake's basal-cut surface and sporadically from the soil's buds. The plant's early development typically dictates the quantity of tuberous roots (Bradbury *et al*., 2013; Schiek *et al*., 2018). Only three to ten fibrous roots (the smallest number) start to bulk up and turn into store roots. Most other fibrous roots become tuberous, but the bulk of them remain thin and continue to perform the role of nutrient and water absorption. Its capacity to take up water and nutrients greatly declines as a fibrous root turn into a store root. Therefore, thin roots penetrate the earth and their enlargement starts only after that penetration. Storage roots are the product of secondary growth of the fibrous roots. The fibrous roots of cassava can reach a maximum of 2.5 m (Adiele *et al*., 2021).

The cassava root cannot be grown vegetatively since it is a true root and not a tuberous root. Three different tissues can be seen on the mature cassava storage root: parenchyma, peel, and bark (periderm). About 85 % of the fresh root's weight is made up of the parenchyma, which is the edible part. It consists of evenly spaced xylem vessels in a cell matrix that carry starches (Byju and Suja, 2020).

The peel layer (which accounts for 11 – 20 % of the root's weight) is made up of cortical parenchyma, sclerenchyma and phloem (Panghal *et al*., 2019). A few cells thick and making up only 3 % of the overall weight, the periderm often loses its outermost parts as the body grows. Root size and form vary depending on the cultivar and the surrounding environment; this variation is larger within a cultivar than it is in other root crops (Ulbrich *et al*., 2021).

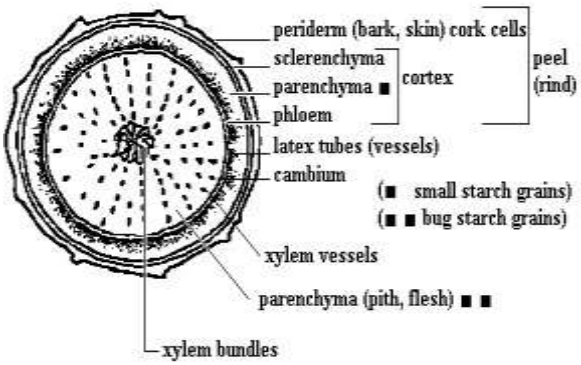


Figure 2.1: Peel Layers of Cassava Plant

Source: Panghal *et al*. (2019)

Roots penetrate the soil deeply despite a low root density. This is an extremely important characteristic because it helps the plant withstand protracted droughts. There are no morphological or anatomical distinctions between fibrous and tuberous roots. Root growth switches from a longitudinal to a radial direction as starch accumulation begins.

However, this does not mean that the root's longitudinal growth has ended. Tuberous roots grow from secondary fibrous root expansion, as was previously stated. This translates to the fact that the root system first breaks through the earth when the roots are sparse, and only then does the root system start to thicken. An adult cassava plant's tuberous portion is visible from the outside, along with its distal end, which may still have a fibrous texture, and its higher or proximal extreme, commonly known as the neck or "peduncle," which also has a fibrous texture and connects the tuberous section to the stem. Neck length might be missing or very short (1 cm) or can be more than 8 cm. The depth at which the stake is buried affects the peduncle's length, and the depth at which the stake is buried tends to make the peduncle longer. Neck length is one aspect that attracts business interest. It damages the tuberous area when it is too short, which speeds up postharvest physiological deterioration (PPD) and inhibits tuberous roots from being separated from their stems.

The peduncle breaks more easily during harvest when it is overly long, leaving roots of interest to the market in the ground and increasing losses (Freschet *et al*., 2021).

Root distribution in the soil is influenced by both cultural and genetic factors. A larger area of soil is covered by types with long necks or peduncles than by varieties with "sessile" roots because their roots are dispersed widely (i.e., with absent or very short necks). How stakes are planted also has an impact on the distribution of roots. The callosity that develops at the lower extreme of a stake when it is planted vertically is surrounded by roots. On the stake, certain lateral buds may produce roots that may eventually turn into tuberous roots. Deeper soil strata are where tuberous roots love to explore and live. Tuberous roots tend to form at the callosity when the stake is positioned at an angle to the soil's surface, and, like in the previous instance, more roots may emerge from the soil's lateral buds. If the stake is positioned horizontally, the tuberous roots are dispersed along the stake because they develop at both the lateral buds and the two ends of the stake. It is also simpler to harvest roots because they are more widely spaced and closer to the ground. The tissue components of a tuberous root are the pulp, peel and central fibers (Ohashi *et al*., 2019).

2.3.1.3 Leaves

Cassava leaves are uncomplicated; they just have a leaf blade, which is created from the petiole and lamina. The palmated veins on the leaf blade are lobed. The number of lobes is generally uneven, ranging from three to nine (occasionally 11). Lobes range in length from 4 to 20 cm and width from 1 to 6 cm. Less lateral lobes than central lobes are present. A lobe's shape can be categorized in numerous ways, with varying numbers of categories. The three varieties of lobes are obovate, straight or linear and pandurate, according to a simple classification. Only a few cultivars have matured vegetative leaves with three lobes, which may represent the primitive ancestral form (Iragaba *et al*., 2019). The leaf

that is closest to the inflorescence's base is usually simple and without lobes, whilst the leaf closer to the inflorescence is typically smaller and have fewer lobes (most often three).

The leaves are alternate and they have a phyllotaxy of 2/5, this implies that any leaf (leaf 1) must circle the stalk twice to get to the sixth leaf (leaf 6). There are five successive intermediate leaves in these two revolutions (with the exception of leaf 1). Cultivars are distinguished by their morphological and agronomic traits and variations, particularly the morphological traits of their leaves, which can change with environmental factors and plant age. Each cultivar is distinguished by its leaf size, albeit this characteristic is greatly impacted by the climate. In comparison to leaves generated after the fourth month, those produced in the first three to four months of the plant's life are larger (taxonomy and morphology). Growing leaves are glabrous, or without pubescence. Two stipules (0.5 - 1.0 cm long) encircle each leaf and continue to be attached to the stem even after the leaf has fully matured (Mueller *et al*., 2014). The usual petiole length of a fully opened leaf is 5 to 30 cm, although it can be as long as 40 cm. Shiny, waxy epidermis covers the upper leaf surface. There is non-homogenous distribution of stomata along the leaves; in fact, the main vein on the upper (adaxial) surface of the leaves has only a few stomata are located while most of the stomata are found on the bottom (abaxial) surface of the leaves (Conklin *et al*., 2019). The adaxial surface of stomata is present on just 2 % of the 1500 cassava varieties (Osadolor and Isese, 2023). It is worth noting that stomata on the upper surface are both larger and more functional. Both exhibit parasitic morphology, consisting of two tiny guard cells encircled by two subsidiary cells (Mueller *et al*., 2014). On a leaf, the area of stomata ranges from 278 to 700 mm^2, and the total area taken up by stomatal pores can be between 1.4 and 3.1 % (Alves, 2001). Most often, photosynthesis transforms solar energy into chemical energy in leaves. The caducous nature of leaves causes them

to deteriorate and shed off the plant as it develops. Environmental conditions have a significant impact on the varietal features of leaves, such as their lifetime and photosynthetic capability, as well as the total number of leaves the plant produces over time (Wimalasekera, 2019).

Another varietal trait is leaf color, though it can change as a plant ages. The color of mature leaves can range from pale green to purple. As the leaves expand and mature, it's possible that purple buds will eventually take on a greenish hue. Due to its consistency, bud color is a particularly useful characteristic for identification of leaf varieties. The nervure's hue, which can also be used to identify a variety, ranges from green to purple. The leaf blade's two sides may have the same color or a distinct one (taxonomy and morphology) (Hasanuzzaman *et al*., 2016).

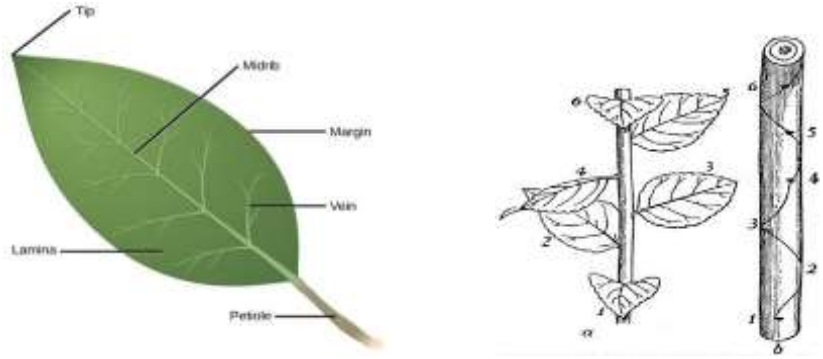


Figure 2.2: Parts of Cassava Leaf and its Arrangement

Source: Hasanuzzaman *et al*. (2016)

The leaves of the cassava produce the starches and protein. Cassava leaves are rich in nutrients, with protein levels ranging from 18 % to 22 % by dry weight (Bayata, 2019). Young cassava foliage also contains a variety of vitamins and minerals. These nutrients serve as the primary components for cell development and growth. As a result, the extent to which the leaves are healthy has a significant impact on yield (Alexis *et al*., 2020).

2.3.1.4 Flowers

Cassava is a monoecious species, which means that it produces both female (staminate) and male (pistillate) flowers in the same plant. Cassava varieties do not all flower in the same conditions. The environment has a significant impact on flowering induction. Inflorescences of cassava produce "flowers" (Pineda *et al*., 2020). Also, there are inflorescences in the axils of the leaf which are located at the upper part of the plant, but they often form at the point where the reproductive branchings inserted. Female flowers are more prevalent on the lower portion of the inflorescence compared to male flowers, which are more abundant on the upper portion. Female flowers on the same inflorescence bloom one to two weeks before male blossoms (protogyny). On various branches of the same plant, male and female flowers may open simultaneously. Cassava is a highly heterozygous plant because it is typically cross-pollinated by insects (Byju and Suja, 2020). The flowers lack a corolla or calyx and instead have an undefined structure known as a perigonium or perianth, which is made up of five reddish, yellow or purple tepals. The male and female flowers differ in size because of few possible factors. Noteworthy, there is a straight, thin and very short pedicel in the male flower, whereas for the female flower, the pedicel is curved, thick and long. A basal disk divided into ten lobes exists within the male flower. Between them, ten stamens emerge. They support the anthers by forming two circles. The five stamens on the outside are divided and longer than the five on the inside, which unite at the top to create a group of anthers. When compared to other flowering plants, the colour of the pollen is often yellow or orange and its size ranges from 122 to 148 m (De Souza *et al*., 2017). The basal disk of the female flower contains 10 lobes as well, but the lobules are less compared to the disk of the male bloom. The ovary is placed on the basal disk and is tricarpellary with six ridges. Each of the three

locules houses one ovule. On top of the ovary lies a little style that gives rise to a stigma with three undulating, fleshy lobes (Ao, 2019).

The raceme, which has male flowers at the apex and female flowers at the base, is the simplest straightforward flower arrangement. In comparison to the females, the latter are often smaller and more abundant. Panicles are regularly generated and, from a botanical perspective, create a raceme of racemes. In these circumstances, a major raceme composed of minor racemes exists. A primary bract and a bracteole are present on both the female and male flowers. These foliaceous organs are seen in inflorescences and often disappear or persist after the development of the flowers. On rare circumstances, the top parts of the plant's inflorescences might develop from buds in the axils of leaves (Reut and Płachno, 2020). As a result of cassava's ability to reproduce vegetatively, reproductive failure is not as harmful to the species as it will be in crops that only reproduce sexually. As a result, it's common to find cases of male sterility, for instance. There are two different kinds of these situations: one in which the flowers fail to mature and the other in which they do but the anthers fail to release pollen. But a complete study of the genetics behind such sterility is still lacking (Ceballos and Hershey, 2017).

2.3.1.5 Fruit

The fruit which has a diameter of 1.0 to 1.5 cm is an ovoid to globate trilocular dehiscent capsule with six straight longitudinal, thin, and noticeable ridges, called aristae. One carunculate seed resides in each locule. The fruit dehisces bicidally, combining septicidal and loculicidal dehiscences, with openings running parallel to the dissepiments and also, along the midveins of the carpels. The fruits open into six valves with this combination of dehiscence, resulting in an explosive dehiscence, thereby forcing the seeds to eject some distance away (Lebot, 2019).

After the female flower is pollinated, fruit starts to develop from the ovary. It takes roughly three months for fruit to mature (Krisanapook *et al*., 2019). Epicarp, mesocarp, and endocarp are the three separate tissues that make up a fruit. With maturation, the seed's epicarp and mesocarp become dry. The ligneous endocarp abruptly opens once the fruit has dried and reached maturity, releasing and scattering seeds a given distance out. Throughout each fruit loculus' mid-vein as well as in between the separations themselves, tissues separate during dehiscence (Ceballos and Hershey, 2017).

2.3.1.6 Seeds

2.3.1.7 General description

An immature plant enclosed in a protective shell is called a seed. Angiosperm and gymnosperm plants, which are classified as spermatophytes and are seed plants, produce seeds as a part of the reproduction process (Nigris *et al*., 2021). Plants reproduce sexually, developing fertilized seeds into ovules as a byproduct. All plants do not generate seeds, but those that do typically rely on these seeds to replicate themselves over successive seasons and years. The matured ovule produces seeds after being fertilized by pollen, the seed then develops within the parent plant. The seed coat will be produced from the zygote, the embryo forms, and the integuments of the ovule (Tan *et al*., 2022).

Gymnosperm and angiosperm plants have greatly benefited from the development of seeds compared to more ape-like plants like mosses, ferns and liverworts, which lack seeds and depend on water-dependent methods of reproduction. Both biologically and economically, seeds are very valuable. Protein, carbohydrates, lipids, and fat reserves, which are plentiful in most plant seeds, are known to help plants grow and develop more quickly in their early stages. These supplies are used by numerous legumes and grains to feed a sizable fraction of the world's population. The majority of biological niches on land

are currently occupied by seed plants, including those in forests, grasslands, and both hot and cold regions (Zhang *et al*., 2022).

There is a definition to seed which is more general and it predates the definitions above: it refers to something that can be sowed, such as "seed" sunflower seeds, "seed" corn, or "seed" potatoes. Sunflower and maize "seeds" are actually the seed inside of a husk or shell, whereas potatoes are actually a tuber (Aravani *et al*., 2022).

Different sized and shaped seeds must fit inside the fruit's outer layer. They can be smooth or corrugated, one colour or multicolored, big or small, and firm or soft. Some require a microscope (orchids for example) to observe because they are so tiny.

The ovary in angiosperms (flowering plants) matures into a fruit that hat houses the seed and acts as a vehicle for seed dispersal (Fenn and Giovannoni, 2021). Many of the "seed"like structures are apparently dry fruits (Pereira *et al*., 2016). Seeds are sometimes enclosed within the fruit's hard wall, that must be cracked open in order to access the seeds. Other modifications vary by plant group; for example, the endocarp of stone fruits (like the peach) surrounds and is bonded to the actual seed. The hard-shelled, singleseeded fruits of some plants, such as acorns or hazelnuts, with an indehiscent seed are known as nuts (Alford, 2007).

A seed is an embryo that develops from a zygote and is a young, immature diploid sporophyte that is encased in a seed coat, encircled by nutritive tissue, and covered in it. The embryo typically comprises of a developing root known as a radicle, the apical meristem of a shoot, known as an epicotyl and one or more cotyledons, which are young seed leaves. The hypocotyls are the transitional tissue between the root and stem. An ovule is an immature seed that has not yet undergone fertilization. There are four stages

in the formation of a seed, from ovule fertilization through physiological maturity (Bareke, 2018). Cell division and growth are the functions of phases I and II.

2.3.1.8 Nutrient storage

Typically, the seed contains nutrients supply for the seed that will develop from the embryo (Vogiatzaki *et al*., 2017). Due to the plant type, the nourishment is stored in a different form. In angiosperms, the tissue known as the endosperm, which is produced by double fertilization from the pollen and the mother plant, serves as the first stage of the food storage (Bareke, 2018). It is typically triploid and high in protein, carbohydrates, or oil. The food-storing tissue (also known as endosperm) of gymnosperms, such as conifers, is a haploid tissue that is a component of the female gametophyte. The peripheral endosperm (aleurone layer), which is loaded with protein-rich aleurone grains, encircles the endosperm (Meziani *et al*., 2021).

The inner endosperm layer was originally known as vitellus, whereas the perisperm was called albumen, similar to the animal ovum. The word started to be used to refer to all nutritional stuff, despite being incorrect. Endospermic seeds are still referred to as "albuminous" in this nomenclature. In relation to the embryonic to endosperm body ratio, this material's nature is utilized to both describe and categorize seeds. Depending on whether or not the cells of the endosperm are farinaceous (or mealy), meaning they contain starch like cereal crops (non-farinaceous). In addition to being described as "fleshy" or "cartilaginous," the endosperm can also be referred to as oily, as in the cases of Poppies, Ricinus (castor oil) and Croton, which have thicker soft cells. According to Roman *et al*. (2017), the endosperm is referred to be "horny" when the cell walls are thick, as in coffee and dates, and when the walls are mottled, as in Annonaceae, nutmeg, and palms, it is referred to as "ruminated".

In many monocotyledons (such grasses and palms) and in some (endospermic or albuminous) dicotyledons (such as castor beans) the embryonic will be implanted in the endosperm (and nucellus), which will be used by the seedling during germination. Moreover, the embryo typically absorbs the endosperm in non-endospermic dicotyledons, as it develops inside the seed, and the embryo's cotyledons fill with nourishment that has been stored. These species' seeds are also known as exalbuminous seeds since they mature without an endosperm. Legumes (including peas and beans), trees (like walnut and oak), radish, sunflowers and squash are examples of exalbuminous seeds. Brazil nuts are stored in the hypocotyl, a location of storage that is unusual among seeds, according to Tiwari (2020), gymnosperm seeds are all albuminous seeds.

2.3.1.9 Edible seeds

Humans mostly get their calories from seeds, especially those found in legumes, nuts and cereals and many seeds are edible (Fuhrman, 2018). The majority of cooking oils, several drinks, spices, and some crucial food additives are also produced from seeds (Martillanes *et al*., 2017). In various seeds, the endosperm or seed embryo predominates and contains the majority of the nutrients. The physicochemical characteristics and amino acid composition of proteins stored in the embryo and endosperm are different. For instance, the gluten in wheat, which is essential for giving bread dough its elastic quality, is only an endosperm protein (Elkonin *et al*., 2016).

Seeds are used for propagation of a variety of crops, such as legumes, cereals, forest trees, pasture grasses, and turfgrasses (Rihan *et al*., 2017). A significant obstacle, especially in developing nations, is the inability of the marketing routes to effectively distribute the seed to disadvantaged farmers (Alemu *et al*., 2019). So, using seed that has been saved by the farmer is still pretty frequent. Animals consume seeds as well (seed predation), and they can be given to livestock or used as bird seed.

2.3.1.10 Oil seeds

The seeds with a comparatively high percentage of oil are referred to as oilseeds. They don't fall under any specific family or classification of blooming plants (Amza *et al*., 2011; Arrutia *et al*., 2020). Groundnut, palm kernel, soy bean, olive, cotton seed, melon, locust bean, castor bean conophor nut, African oil bean, rapeseed, sunflower seed, safflower, sesame seed, linseed, and other similar seeds are included in this category. Some of these seeds are currently underutilized compared to their potential since they are not widely known. Some of them have been discovered to be extremely nutrient-dense as nuts and wild forest seeds (Enujiugha, 2017).

Modern research efforts are focused on utilizing the nutritious prospects of some of the less-known oilseeds in developing nations where supply protein from animal sources cannot meet the protein demands of the rapidly increasing population. Because many of such these seeds are protein-rich, they can be utilized to fortify different starchy staples and create new items that will enhance peoples' protein intake. Examples include the creation of a shelf-stable and an acceptable bread-spread from African oil bean seeds and a biscuit made from conophor nuts (Sunil *et al*., 2015). Most of the less-known oilseeds are cooked and then eaten, either as soups or as snacks and sauce toppings after being fermented (Mall, 2017).

2.3.1.11 Functions of seeds

The plants that produce seeds use seeds for a variety of purposes. These include hibernation during poor conditions, dispersal to a new place, and nutrition of the embryo. Fundamentally, seeds are a form of reproduction, and the bulk of seeds are produced sexually, resulting in a mixture of genetic material and phenotypic variability that is subject to natural competition. Endophytic bacteria found in plant seeds have a variety of uses, but disease resistance is one of their most crucial ones (Liu *et al*., 2020).

1) Embryo nourishment

Embryos and young plants are nourished and protected by seeds. As a result of the higher food stocks in the multicellularity of the encapsulated embryo and the seed, they typically provide a seedling with an earlier start than a sporeling from a spore (Baroux and Grossniklaus, 2019).

2) Dispersal

Plants can only find a limited number of optimal conditions for survival and growth, unlike animals. Plants have therefore developed a variety of strategies for distributing their seeds in order to reproduce (vegetative reproduction). A seed needs to "arrive" where it is going and be present at the appropriate time for growth and germination. Dehiscent fruits, which include, follicles, capsules, siliques, legumes and silicles, open and expel their seeds on a regular basis are characteristic of related groups of plants. Caryopses, achenes, nuts, utricles and samaras are examples of indehiscent fruits, which are those that do not regularly open and eject their seeds (Singh *et al*., 2019).

2.3.1.12 Other Uses of seeds

1) Cotton fiber is made from cotton plant seeds. Kapok and milkweed seeds also yield other seed fibers (Panahi *et al*., 2020).

2) From seeds, significant non-food oils are derived. In paintings, linseed oil is being utilized. Jojoba and crambe oil resemble whale oil (Erhan and Adhvaryu, 2020).

3) Some medications, such as tea tree oil, castor oil and the bogus cancer treatment Laetrile, are derived from seeds (Schwarcz, 2014).

4) Castor bean, chinaberry, rosary pea and job's tears are just a few of the seeds that are used as beads in rosaries and necklaces. The first three, though, are also poisonous (Patel *et al*., 2017).

5) Cottonseed meal is utilized as fertilizer and animal feed (Estupiñan-López *et al*., 2017).

2.3.2 Cassava seed

Cassava (*M. esculenta*), which does not generate a lot of sexual seeds, it is vegetatively grown by cuttings of mature stem (stakes). On the other hand, wild species are spontaneously multiplied by seeds (Alves *et al*., 2014). Within the lignophytes, cassava seed plants constitute a monophyletic lineage. In some varieties of cassava, such the bitter cassava, flowering occurs frequently and regularly. The flowers are carried on the terminal panicles, which share an axis with the panicle inflorescence. Insects use flowers as a means of pollinating crops. The immature fruit emerges from the cassava ovary during pollination and subsequent fertilization. After pollination, the fruit must mature for three to five months. The fully developed fruit is a globular, 1 to 1.5 cm diameter capsule. Three woody lobules on the endocarp, each bearing a single seed, make up the endocarp. When the fruit reaches maturity, it dehisces or explodes (Subramaniam *et al*.,

2022). When fully developed, the capsules turn dark and explode, releasing the tiny seeds (Popoola and Yangomodou, 2006). Cassava seeds are smaller than castor oil (*Ricinus communis*) seeds but are similar in size and form. Since the stems are planted vegetatively to grow new plants, they are only agricultural waste.

The approximate dimensions of a cassava seed are; 100 mm long, 6 mm wide, and 4 mm thick. They have an ovoid-elliptical shape. Each seed weighs between 95mg and 136 mg (Ceballos *et al*., 2012). The coat of the seed is silky, dark brown and flecked with gray. Within 16 days of being collected, the seeds often begin to germinate. While not crucial for economic multiplication and reproduction, it is of enormous worth for plant breeding because only reproduction sexually allows for the creation of novel, genetically superior cultivars. It has an ovoid-ellipsoid shape. Coffee-colored, Smooth and speckled grey describe the seed coat. The caruncle is located in the top portion, particularly of fresh seed. From the caruncle, a thin suture emerges and terminates in the basal hollow. Figure 2.3 depicts the normal structure of a cassava seed. The seed's outermost layer is called the seed coat. The endosperm, composed of tetrahedral parenchymatous cells and is immediately inside the seed coat, protects and nourishes the embryo, which is situated in the center of the seed. The embryonic and cotyledons axis which will produce the new plant following germination are located within the endosperm. The hypocotyl, plumule, the two cotyledonous leaves and radicle make up the embryo. The majority of the seed's interior is made up of the white, elliptical, and carnose cotyledonous leaves and endosperm. Even though seed doesn't now dominate cassava proliferation, it very well could later. Apomixis is a process that occurs in nature, particularly in grasses, and refers to the formation of botanical seed without the customary sexual reproduction. In other words, apomixis results in seed embryos that are identical genetically to the parent plant. This implies that the embryo will create a unique plant that is similar to its mother as it

develops into a plant. The Manihot genus has been associated with apomixis (Nassar, 2000). Due to its major benefits, which include the ability to store seeds for longer than the month or two that stems can be stored and the ability to significantly boost the rate of replication of a material, it might be included into commercial systems. In comparison to stem cuttings, they are also less likely to harbor infections.

Manioc seeds have substantially more protein than the tubers, that are one of the main sources of both carbohydrates and proteins in underdeveloped nations (Nartey *et al*., 1974). The endosperm and cotyledon of mature cassava seeds both contain proteins and lipids as significant reserves, according to electron microscopic investigations (Nartey *et al*., 1973). It was recently revealed that the kernels of cassava seeds contain 34 % proteins and 47 % lipids, and was hypothesized that these seeds could be a significant economic and nutritional source of both proteins and fats. Cassava seeds contain 34 % protein, demonstrating that they are comparable in protein content to leaves. When processed, these seeds may produce a meal with up to 64 % protein (Nartey and Møller, 1976). In developing nations where the plant is widely cultivated, cassava seeds might be a valuable resource both commercially and nutritionally.

According to research, 57 % of the *M. esculenta* seed is made up of the seed kernel. which are electronmicrographs of the hypocotyl/radicle tissues and endosperm/cotyledon of *M. esculenta*, demonstrating the presence of protein bodies, large lipid globules and other ultra-structural units. The endosperm makes up 96 % of the seed kernel and it contains the majority of storage forms of proteins and lipids, though there are similar pattern of storage materials in the embryo tissues. The lipid content of *M. esculenta* seed kernels are abundant. According to analysis, lipids made up 47 % of the dry kernel weight, which constituted the main seed storage reserve. only 0.1 3 %. Only 0.08 % of the phosphates

were inorganic, but there were 1.36 % of organically bound phosphate, which made up the majority of the remaining phosphates (Nartey *et al*., 1974).

2.3.2.1 Cultivation of cassava seed

The most popular method for domestic (subsistence) consumption and commercial output is vegetative propagation via stem cuttings. Although actual botanical seeds can be used to propagate cassava, this approach is hardly ever used. shortage of high-quality planting material for smallholders is caused by practical issues such as the phyto-sanitation, perishability and bulkiness of cassava stem cuttings (Nduwumuremyi *et al*., 2016; Parmar *et al*., 2017). Another difficulty is determining the rates of multiplication; for instance, one maize seed can yield 300 seeds in three months, but only 10 stems from one cassava stem in a year (Howeler *et al*., 2013). The International Institute of Tropical Agriculture (IITA) developed a quick-multiplication technique termed "mini-stem cuttings" that allows 60 to100 mini stem cuttings for each plant and as few as one bud for each stalk. When root production is the main goal, a plant density of roughly 10,000 plants for each hectare is ideal (i.e. one plant for every square metre).

Cassava stalks can be grown vertically, horizontally, or inclinedly, due to the type of soil and agro-ecological circumstances. Horizontal planting is advised in regions with little rainfall (1000 mm), however vertical planting may be an option in sandy soils with abundant rainfall. It is thought that planting vertically or at an angle result in larger yields. Depending on the weather and type of soil, the planting depth might range from 5 to 15 cm. Stalks should be planted deeper on sandy soils in dry places to prevent loss of moisture. Although a hardy crop, cassava cannot withstand weed competition and water logging (high soil moisture) and during the first stages of development. Therefore, it is necessary to prepare the soil adequately for good drainage, aeration, and weed control

(Lebot *et al*., 2009; Howeler *et al*., 2013). Cassava farms can be designed as flat till, notill, mounds and ridges depending on the edaphic conditions. Due to the abundance of organic matter and the absence of compaction, Amazonian Indians frequently planted cassava stalks without tillage. Ridges and mounds are frequently formed under rather poor edaphic conditions. According to FAO's "Save and Grow" enhanced cassava farming techniques, mulch or ground cover can prevent soil surface erosion and help weeds grow less (Howeler *et al*., 2013; Parmar *et al*., 2017).

2.3.2.2 Germination of cassava seed

At room temperature, cassava seed germinates irregularly over a period of 2-4 months. The germination rate is typically between 10 and 40 % (Hahn *et al*., 1974). Historically, "breaking" the seed coat was advised and resulted in 80 – 100 % germination in 15–20 days. However, because the embryo was damaged during the cracking process, this procedure was highly arduous and many of the seedlings were subpar. The Federal Experiment Station of Nigeria has created a highly effective wet-heat treatment that allows a large number of seeds to germinate in a uniform stand in a short amount of time.

lITA uses unscarified seed, which is sprayed with an insecticide and fungicide before being planted in shallow drills and kept well-watered in the nursery in January/March. A three-week germination rate of 80 % is possible because the soil temperature is within the necessary range at this time of year, and an irrigation schedule assures sufficient moisture. In 1972, 12,000 seedlings were generated using this method, and in 1973, 100,000 seedlings were produced. This method's overabundance of weeds is its only drawback. Pre-planting treatments were tested in an experiment to shorten the time between field planting and seedling emergence and to lessen weed competition. Within nine days of planting, a pre-sowing procedure that involved placing sieved soil in wooden flats that

were kept outside and well-watered led to a uniform germination rate of 85 %. Planting untreated seeds in deat pots held in wooden seed trays or black plastic seed trays is a practical and effective (germination rates of 80 – 90 %) method for modest studies. These grow just as well outside or in the warmer screen homes if they are maintained properlywatered. A number of investigations on seed germination have shown that light and abrupt temperature changes are not necessary for proper germination, but that a soil temperature between 30 and 35 ^{o}C and consistent moisture are (Hahn *et al*., 1974; Ferguson *et al*., 2019).

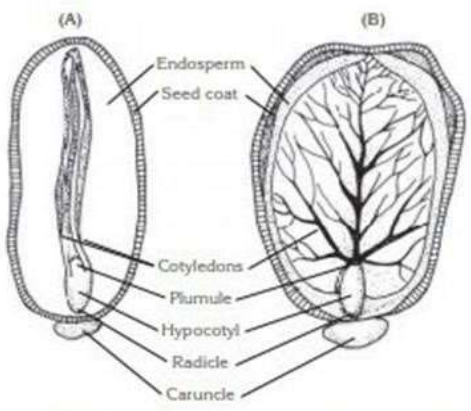


Figure 2.3: Diagram Showing Two Longitudinal Segments of Botanical Cassava Seed. (A) Cross-Segment cut across Suture; (B) Cross Segment cut through Suture.

Source: Hahn *et al*. (1974)

2.4 Classification of Cassava

Cassava comes in a variety of cultivars, and they can be identified from one another based on a variety of structural characteristics. Other traits used to distinguish the many plant kinds include tuber shape, early maturity, yield, and its presence of cyanogenic glycoside. According to reports, two edible species, Sweet (*Manihot utilissima Phol*) and Bitter (*Manihot aipi Phol*), have high and low cyanogenic glucoside contents, respectively

(Nnamani and Okonkwo, 2017). Cassava has a ploidy number of 2n = 36. The tropical and subtropical Americas are home to numerous closely related species that can interbreed with *M. esculenta* (Simon *et al*., 2022).

Cranzt originally gave the scientific name for cassava in 1766. Then, based on whether it was bitter (*M. utilissima*) or sweet (*M. aipi*), it was reclassified as 2 distinct species (Ceballos *et al*., 2012). But an Italian by the name Ciferri Raffaele acknowledged and proposed that the cassava's current name of *M. esculenta*, should be given precedence for the scientific nomenclature (Rogers and Fleming, 1973). Three subspecies of the species *M. esculenta* were suggested by Allem (1994): Esculenta, *M. esculenta* subsp. subsp. Peruviana and subsp. flabellifolia (Simon *et al*., 2022).

There is no evidence that the classification of cassava cultivars as "sweet" or "bitter" correlates with phenotypic features (Ospina *et al*., 2021; Ogbonna *et al*., 2021).

Botanists have found it to be essentially difficult to categorize these kinds according to their relative toxicity. This regional and cultural pattern of distribution would appear to indicate that the distinctions between the sweet and bitter varieties of manioc are influenced by either cultural or geographical factors in selection (Mühlen *et al*., 2019).

Cassava is classified as sweet or bitter (*Manihot utilissima* or *Manihot palmate*, respectively) according to the possibility that acute symptoms will occur following consumption of the product if extra care is not exercised to detoxify it (Nathan and Udosen, 2017). Some articles describe bitter cassava (high cyanogenic potential) as *M. esculenta*, while sweet cassava (low cyanogenic potential) is classified as *M. utilissima* (Boakye Peprah *et al*., 2020).

Frequently, it is described as "sweet" or "bitter" based on the cyanide level, however these descriptions are at best approximations, and attempts to link cyanide levels with specific botanical taxa are extremely false (Lu *et al*., 2020). It is impossible to make a direct connection between sweetness and bitterness in flavor (Wang *et al*., 2018).

Bitter cassava has a high cyanide content, while sweet cassava has a lower value, but there is a lot of overlap between the two classes. Bolhuis (1954) demolished much of the mythology that had grown up around the subject of cassava toxicity and bitterness Aside from cyanogenic glycoside content, the factors responsible for sweetness or bitterness require further investigation.

Manioc (Euphorbiaceae, *Manihot esculenta*), a cyanide-containing food crop, which was first grown in the tropical forests of Central and South America, has a long tradition of being divided into "sweet" and "bitter" (non-toxic and toxic) types. But there is no taxonomic basis for this distinction. In addition to being extremely variable, the amounts of cyanogenic glucosides in different manioc types also don't match up with any other morphological or ecological characteristics. However, it is frequently asserted that these two "varieties" have different cultural and geographical distributions, and are each connected to a specific local food complex (Delêtre *et al*., 2021).

2.4.1 Sweet cassava

It was recorded that sweet manioc had a different geographic distribution than bitter manioc and required considerably easier native processing methods (Alves-Pereira *et al*., 2018; Sauer, 2017). Since then, there has been extensive research on the types and consequences of the toxins present in bitter manioc (Amala, 2016; Dufour *et al*., 2016; Imakumbili *et al*., 2019).

It has been well documented that bitter and sweet manioc appear to be distributed into specific regions of South and Central America (Mühlen *et al*., 2019; Iriarte *et al*., 2020), and that the labour-intensive processing techniques associated with bitter manioc have been described extensively by ethnographers (Bulkan, 2019). The contemporary ethnographic and historical literature, however, largely sticks to the initial perceptions and observations surrounding bitter and sweet manioc.

They can be also categorized as short-season chorionic villus sampling, which can reach maturity as early as six months after sowing and can't be left in the soil for more than nine to eleven months without significantly deteriorating. According to Rakotoarivony *et al*. (2022), the sweeter types may be more vulnerable to harm by some mammals and wild pigs, however it has not been scientifically confirmed. When cassava only makes up a minor portion of a diverse crop system, "sweet" varieties are most commonly grown exclusively (Macfadyen *et al*., 2021). In almost all regions where cassava is the primary crop, "bitter" cassava cultivars predominate and "sweet" varieties are planted in much smaller quantities or not at all (Nakabonge *et al*., 2018).

2.4.2 Bitter cassava

Bitter cassavas are typically the major staple where they are farmed (Bulkan, 2019). They can also be classified as long-season chorionic villus sampling, which are often bitter cassavas that require a minimum of one year to bear fruit and can even survive in the ground for up to four years (Adamu, 2014).

Bitter varieties are said to deteriorate more slowly (Bechoff *et al*., 2018), but there is no evidence to back this up. Even the relationship between sweet varieties and shorter cultivation cycles may be influenced by environmental effects and cultural practices, given that there have been several evidences that the roots become much more bitter

merely by being left in the soil longer and sweet cultivars could be pulled earlier for reasons unrelated to their inability or ability to remain in the soil (Imakumbili *et al*., 2019).

The "bitter" varieties must undergo specific processing before consumption as they are poisonous (Bolarinwa *et al*., 2016). This classification system might help in explaining the origin of bitter manioc's association with slower and even more involved processing systems.

The "bitter" cultivars with a high cyanogenic glucoside concentration predominate almost everywhere where cassava is an important staple crop (Imakumbili *et al*., 2019; Deng *et al*., 2021). If these substances aren't processed down to negligible levels, they might have negative effects (Senica *et al*., 2016; Kunatsa *et al*., 2020).

Bitter cultivars are often preferred by farmers because they prevent animals, thieves and pests (Airaodion and Ogbuagu, 2020).

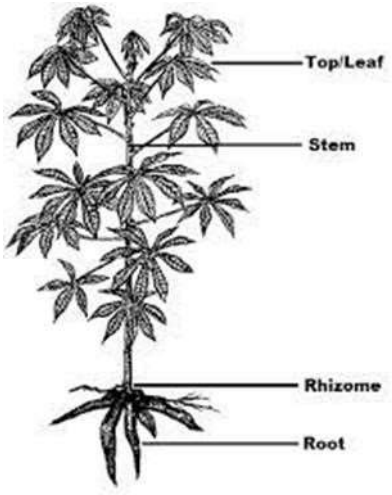


Figure 2. 4: Cassava Plant

Source: Raji *et al*. (2016)

2.5 Nutritive Value of Cassava

Over 500 billion people in the developing world eat cassava as a staple meal as part of their daily diet (Mombo *et al*., 2017). The use of industrial starch in products like animal feed, fresh foods with a starch basis, and other commodities is now well-established in commercial agriculture.

In the tropical regions, cassava is the third largest source of carbohydrates and, it is Africa's fourth largest source of human energy and is superior to other starchy crops in terms of its capacity to accumulate starch, tolerance to drought, and resistance to low soil nitrogen levels (Cosman *et al*., 2016; Nathan and Udosen, 2017; Li *et al*., 2017).

Additionally, cassava is a suitable source of energy for both industrial biofuel usage and human consumption since it contains 80 % starch and is high in carotenoids, minerals and vitamin C. Furthermore, it contains glucose, nicotinic acid, thiamine, and riboflavin (Joshi, 2020).

Being a tropical crop, cassava is very low content of crude protein compared to grainss and high in energy and crude fiber (Bayata, 2019). In many states, cassava is a vital component of the diets of both urban and rural residents. It is utilized in the industrial sector and for production of animal feed (Nadjiam *et al*., 2020). Zhu (2015) have shown that cassava tubers have a very diverse range of physicochemical compositions in terms of nutritional value and that cassava cultivars in the Amazon region have a lot of free sugars (Laya *et al*., 2018).

These cultivars are ideal for fermentation and the commercial production of ethanol and organic acids (Oladeji *et al*., 2019). In contrast to their high carbohydrate and up to 35 % starch content, tubers have a very low protein content (1.1 %) (Hohn and Nell, 2017).

There are new hybrid cultivars with tubers that contain up to 5 % protein (Esuma *et al*., 2016). Nonetheless, the leaves are higher in protein (more than 25 %) and contain a wide range of minerals and vitamins (Koubala *et al.*, 2015; Agnoli *et al*., 2017; Melesse *et al*., 2018).

In general, the cultivars, their age, cultural practices, and climatic circumstances all affect the physicochemical makeup of the leaves and tubers (Adegbenro, 2018). The use of cassava root to replace more than 50 % of the corn in poultry diets is generating significant interest. The leaves of the cassava plant are high in nutrients, low in energy, high in crude protein, and a strong source of protein and beta-carotene. Broilers and layers can both withstand inclusion levels of 20 %. Cassava peels, which make up 15 % of the entire cassava root yet may be considered an environmental nuisance, can be utilized in chicken feed. Compared to the cassava root, it has higher levels of HCN, crude fiber, and lower levels of protein and calories. The protein level of cassava tubers ranges from 0.7 - 1.3 % fresh weight (Adedokun *et al*., 2019). Cassava leaves, peels and flour have low protein contents as well, at about 3.6 %, 5.5 %, and 21 %, respectively (Morgan and Choct, 2016). The majority of the carbohydrates in the root are readily available. Cassava roots contain lower protein (2 - 3 % compared to 8 - 10 % for maize) than other energy sources like maize. When cassava flour is used to make animal feed, it costs roughly 70
% more than maize flour because of the difference in protein content (Latif and Müller, 2015). Cassava protein is abundant in arginine level but deficient in isoleucine, methionine, cysteine, threonine, phenylalanine, proline, and other amino acids (Raji *et al*., 2021). Lipid content in cassava is very low. Bayata (2019) discovered that maize possesses about 6 % of lipids, but cassava has barely 0.1 %. Cassava flour contains about 2.5 % lipids, however traditional solvent systems can only extract half of this, and the fatty acids in cassava are mostly saturated. Cassava has high levels of vitamin C but low

levels of vitamins A, B1, B2, and niacin due to its low lipid content, making it a poor source of fat-soluble vitamins (Morgan and Choct, 2016).

2.6 Uses of Cassava

Cassava can be used in a variety of ways. Agriculture, medicine, automobiles, packaging, and drug release are a few examples. Both the industry and the public are becoming more conscious of their environmental responsibilities. Cassava roots are utilized in many different ways, such as starch and food for human and animals.

2.6.1 Animal feed and industrial use

cassava is widely employed as animal feed and as a raw material in industries, in addition to being a significant staple meal for millions of individuals. Most cassava exporting countries, including Vietnam and Thailand, produce silage (roots and leaves) and cassava pellets for animal feed.

The cassava roots are first dried in the sun, then mechanically peeled, chipped, and processed into pellets for export. Animal feed made from cassava is supplemented with fish meal or soybean meal in order to increase the protein level and provide balanced diets to livestock. Cassava meal has great digestion because it contains yeasts and lactic acid bacteria that are found in nature. According to Parmar *et al*. (2017), tiny quantities of hydrogen cyanide (HCN) found in cassava increase the effectiveness of lactoperoxidasse, a natural antibacterial enzyme released by the breast, mucosal, and salivary glands.

One of the most affordable sources of starch is cassava, the extraction of starch is also simpler because there aren't many soluble carbohydrates present. In addition to bioplastics, lactic acids, fructose glucose, dextrin, baker's yeasts and bioethanol, flour and starch are used as raw materials for a variety of industrial products (Parmar *et al*., 2017;

Kadier *et al*., 2021). Cambodia, Thailand, China and Vietnam, are the top processors and producers of cassava starch into a variety of products including fuel ethanol and modified starch (used in the paper textile, and plywood industries).

2.6.1.1 Industrial uses of cassava

Cassava can be cultivated in many agricultural ecologies, including in soils that are only marginally fertile, due to its drought tolerance. This allows for flexible, low-cost vegetative multiplication during various harvesting seasons and times (UchechukwuAgua *et al*., 2015). Cassava is an important industrial crop industrial applications and food security because of these agronomic qualities (Jarzebski *et al*., 2020).

2.6.2 Traditional uses of cassava leaves

The starchy roots of the cassava plant are its main byproduct, while some African, Asian, and South American nations also eat the leaves as a vegetable. Cassava leaves are readily available all year long and are high in minerals (K, Ca and Mg), vitamins (carotenoids, B2, B1and C) and protein. According to Latif and Müller (2015), about 20 Sub-Saharan African nations, from Senegal down to Madagascar, as well as four Asian nations (Sri Lanka, the Philippines, Malaysia and Indonesia) consume cassava leaves in a variety of ways. One of the most popular methods of preparation is to boil the leaves for a number of hours, followed by pounding or chopping, and seasoning with other ingredients and spices that are readily available in the area. Currently, Congo is the country that consumes the most cassava leaves. Approximately 60 % (85,000 tons) of the total vegetables consumed in Congo in 2013 were made up of cassava leaves (Parmar *et al*., 2017). In the past, several studies have suggested using cassava leaves in economically underprivileged areas in tropical nations to supplement diets centered on cassava roots (Bechoff, 2018; Alamu *et al*., 2021).

Additionally, cassava leaves are dried in the sun and ground into flour for later usage. In Northeastern Brazil's national food supplement program for low-income communities, flour made from cassava leaves has been combined with grains like wheat and maize to prevent child malnutrition (Parmar *et al*., 2017; Alamu *et al*., 2021). Intake of cassava leaves may reduce in naturally when other vegetables are abundant due to its reputation as a poor man's diet.

However, it might be a significant part of people's diets during times of emergency. Although cassava leaves are rarely eaten under normal conditions, they were commonly devoured in the eastern areas of Nigeria during the Nigerian Civil War (Ubalua *et al*., 2016).

2.6.3 Textile industry

Starch is necessary in the textile industry to size cotton yarn before weaving. Cotton fiber is converted into yarn with various counts (ranging from 0 to above 80) for the creation of various cloth types, ranging from coarse cloth for use in creating towels, dhotis etc. to fine cloth used to make materials for dress. When it comes to size, maize starch is the main rival of cassava starch. Cassava starch was favored for sizing coarse yarn, which is defined as counts from 0 - 40 and maize starch was preferrable for sizing fine yarn, which is defined as counts from 40 and above. These sizing industries were found in the Somanur area, close to Coimbatore and Ichhi-lakaranji. The production of cotton yarn increased by 4 % over the past 20 years. Due to the change in consumer spending from clothing to other durable goods, the textile sector is currently stagnating. The usage of cotton to synthetic fabrics has changed over the last 20 years within India, as seen by the ratio of cotton to synthetic fabrics, which is currently 70:30 compared to 90:10 in the 1980s. Leaving aside these unfavorable aspects, a bright image for the cassava starch demand in the textile industry may be seen when considering the predicted availability of cotton cloth

per person and the healthy growth pattern in the making of cotton yarn over the past 20 years. 10 to 12 % of the mass of yarn that has been sized is made up of sizing ingredients. The key basic elements utilized during the size of yarn are binder, starch (cassava or maize), wax, oil softener and water. There are over 176105 looms in the country, and 3,500 size units dispersed across the nation were able to satisfy their needs. There is no evidence that India has any size units. An estimated 50,000 tons of cassava starch are currently being used in the cotton yarn sizing sector. According to the report, the size industry would need 69,208 tons of cassava starch by 2010–2011 and 78,253 tons by 2015–2016 (Breuninger *et al.*, 2009).

2.7 Challenges of Cassava Utilization

Cassava has a promising nutritional profile; however, it is thought to have endogenous antinutritional elements that could reduce its nutritional value. However, a variety of problems, including the high content of fibre, the presence of anti-nutritional elements and low energy content, especially hydrocyanic acid (HCN), limit the use of cassava.

Its toxicity is a significant issue that restricts the use of cassava as diet. Two cyanoglucosides (CNG) found in cassava, lotaustralin and linamarin is degraded by the enzyme linamarase to release the poisonous compound HCN. This reaction happens naturally in plants to break down plant tissues or in an animal's digestive system. In root peel, there is a particularly strong generation of hydrocyanic acid. There is cyanogenic potential in more cassava plant tissues, including the leaves. The environmental conditions in which a cassava plant is grown and its maturity level at harvest have a significant impact on the cyanogenic capacity of various tissue.

A specific cultivar's roots may taste sweet in one region but bitter in another. However, throughout various tests, the cyanogenic potential of bitter cultivars has consistently been found to be higher (up to 1000 milligrams of acid for each kilogram of fresh roots)

compared to sweet types (20 mg/kg of root). Evidently, there are no cassava types that are cyanogen-free (McMahon *et al*., 2022).

Pathological disorders have been linked to consumption of cassava leaves (Achidi *et al*., 2008). This is caused by the presence of linamarin, lotaustralin, and cyanogenic glucosides in cassava. These glucosides produce quantities of HCN that range from moderate to fatal, and which is a potent inhibitor of enzyme-catalyzed processes, particularly the cytochrome oxidase systems involved in respiration (McMahon *et al*., 2022). The "cyanide scare" is the main issue with the widespread consumption of cassava leaves as food in Nigeria because, depending on the type, the leaves' level of cyanogenic glucosides may be six times higher than that of the roots. Cassava leaves' nutritional value may be constrained in addition to cyanide by tannin and perhaps phytin (Fasuyi, 2005). Cassava is typically processed prior to use in order to reduce toxicity and improve palatability. Several traditional methods of processing cassava have been developed. The processes include cassava tuber fermentation, maceration, boiling, soaking, roasting and other variations. Cassava, particularly bitter cassava, can be used if properly processed to reduce its high cyanide content.

2.8 Plant Food Nutrients

2.8.1 Carbohydrates

Carbohydrates are among the four main types of macromolecules. They have a variety of significant functions in all kinds of life. They act as energy reservoirs and fuel sources. They are also fundamental components of many lipids and proteins (glycolipids and glycoproteins), noteworthy, they are vital to cell-cell recognition and molecular targeting in cell membranes. They are structural elements of plant cell walls, portions of DNA and RNA in which deoxyribose and ribose respectively, are linked to purine and pyrimidine bases by

N- glycosidic bonds, and parts of both RNA and DNA (Danne *et al*., 2017). Depending on their molecular size, carbohydrates can be divided into many different types of molecules. Polysaccharides, oligosaccharides, and monosaccharides are the three main size classes of carbohydrates. Monosaccharides or simple sugars, are made up of ketone molecule or a single polyhydroxy aldehyde. The 6-carbon sugar D-glucose, which is sometimes referred to as dextrose, is the most prevalent monosaccharide in nature. Short chains of monosacharride units, or residues, make up oligosaccharides. The peculiar linkages between these chains are called glycosidic bonds. The most common kind of carbohydrates are disaccharides, made up of two monosaccharide molecules. An example of a disaccharides is sucrose (cane sugar), it is composed of the 6-carbon sugars Dfructose and D-glucose. Polysaccharides are sugar polymers, they are made up of include 20 or more monosaccharide units. Others, like glycogen, are branched; some

polysaccharides, like cellulose, are linear chains (Gopinath *et al*., 2018).

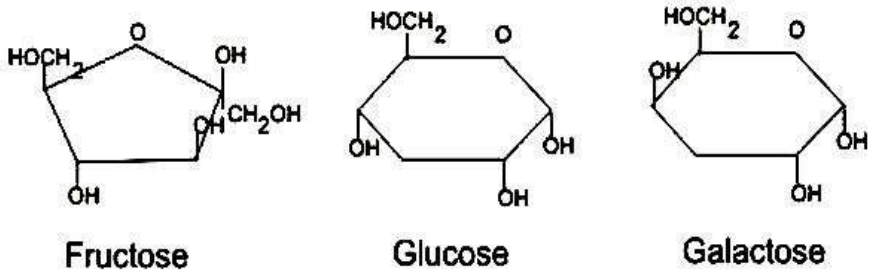


Figure 2.5: A Structure of Monosaccharides

Source: Gopinath *et al*. (2018)

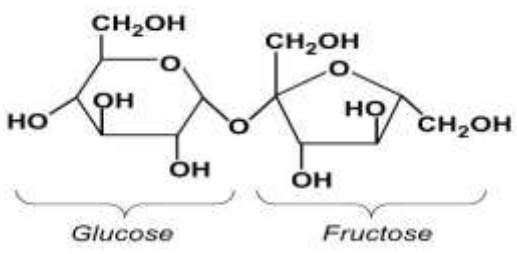


Figure 2.6: A Structure of Sucrose, a Disaccharide

Source: Gopinath *et al*. (2018)

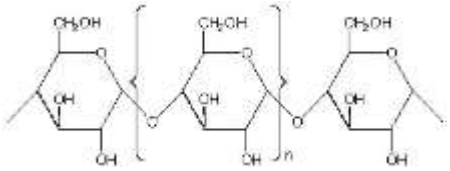


Figure 2.7: A Structure of Polysaccharide

Source: Gopinath *et al*. (2018)

2.8.2 Proteins

Protein is the dietary component that contains the highest nitrogen in both the body and the diet. It is among five groups of complex biomolecules that are found in cells and tissues, along with lipids, polysaccharides, RNA and DNA. L-amino acids polymerization via peptide bond formation aids the creation and structural framework of proteins. These can be made up of two or even more polypeptide chains that come together to create multimeric proteins; the individual chains are known as subunits. The workhorses of cells and organs are proteins, which are made up of amino acids that are put together in the DNA base sequence's prescribed order. All secretions, including peptide or protein hormones and digestive enzymes, are necessary since proteins are important components of all cells. Plasma proteins, which use also used for osmotic balance maintenance of substance transported through the blood, and for immunological function, are synthesized from amino acids found in dietary proteins. Protein surplus is used for energy, glucogenic amino acids can be converted to ketogenic amino acids which glucose can converted to fatty acids (Marczuk *et al*., 2018). If carbohydrate and fat supplies are sufficient to meet energy demands, both types of amino acids are transformed to triacyglycerol in adipose tissues (Tanaka *et al*., 2017). Since the human body lacks a protein storage facility, dietary

protein must be eaten with each meal; nonetheless, a portion of dietary protein is constantly broken down and resynthesized. The degradation of this protein is accelerated while fasting, and the resulting amino acids are used to produce glucose, nitrogenous substances that are not proteins, and the crucial secretory and plasma proteins. Some amino acids are also used in the fed state to provide energy and serve as biosynthetic precursors (D'Este *et al*., 2018).

The most typical type of malnutrition is protein energy malnutrition, which is more prevalent in underdeveloped nations. Protein and energy deficiencies in newborns and young children lead to protein and energy malnutrition. Protein energy malnutrition occurs in two forms: Marasmus and Kwashiorkor. Kwashiorkor is brought on by a deficiency in energy and protein, whereas marasmus is caused by an insufficient intake of protein and sufficient intake of energy. Because of a lack of T lymphocytes, faults in the development of phagocytes and deficiencies in the synthesis of immunoglobulins, interferons and also other components of immune system, children who are protein energy malnourished have stunted physical and mental development and have a weaker immune system. Instead of famine, many of these children pass away from secondary infections (Vishwakarma and David, 2021).

2.8.2.1 Protein quality

The nutritional value of food proteins for maintenance and growth is predicted using protein quality assessments. Biological (*in vivo*) experiments evaluate protein metabolism and utilization by measuring nitrogen balance or growth. Protein quality assays are meant

to indicate protein digestibility, amino acid composition and amino acid bioavailability of the food constituents under investigation. Data on amino acid content is frequently compared with reference proteins (Gaudichon and Calvez, 2021).

2.8.2.2 Method of estimating protein quality

The amino acid score, a metric for assessing protein quality can be calculate from the amino acid composition of the test protein. Amino acid compositions of test and reference proteins are compared. Protein quality is often estimated by determining the level either the first limiting amino acid or the amount of all the essential amino acids. This estimate could be adjusted for digestion based on an *in vivo* or *in vitro* assay. In analyses based on amino acid composition, the amino acid found in the test protein is compared to corresponding levels of human milk, milk protein, egg protein or any other reference pattern, noteworthy, all comprises are based on the amino acid requirements of humans. When determining the nutritive value of a specific protein for adults, older children and infants, different reference patterns are employed because the requirements for amino acids change with age. Despite the possibility that this may overestimate protein needs and underestimate protein quality for older children and adults, the reference pattern which was generated for preschoolers is recommended for determination of amino acid score for all groups except newborns (Malla *et al*., 2022).

The amino acid score of each essential amino acid is the ratio of amount of each essential amino acid in a particular test protein to the corresponding amount in the reference protein. The amino acid which has the lowest score among the amino acids is the first limiting amino acid. There are two methods for calculating amino acid scores: either a ratio in relation to a percentage or the standard.

Amino acid score = mg of amino acid in 1g of test protein (mg/g) (Nielson, 2001) …..(1)

amino requirement for preschool children (mg/g)

2.8.3 Lipids

Lipids are organic substances with a substituted carbon backbone made of oxygen and hydrogen. Additionally, sulfur, nitrogen and phosphorus are included in certain lipids. The majority of lipids differ from proteins and carbohydrates in that they are somewhat but not completely insoluble in water. Since some complex soaps, lipids and short- to medium-chain fatty acids are soluble in water, though some exceptions exist. Simple, derived, compound (complex) and other lipids can all be grouped together. Simple lipids are products of esterification between fatty acids are some alcohols, such as glycerol or cholesterol (Punyu, 2015). They are made up of vitamin A and D esters, waxes, cholesteryl esters and triacylglycerols (natural fats and oils). For compound lipids, they are esters of fatty acids that have been combined with alcohols and other groups, examples of these compound lipids are: lipopolysaccharides, cerebrosides glycolipids, lipoproteins, sulfolipids and phospholipids. Derived lipids are the byproducts of the hydrolysis of complex or simple lipids, such as diacylglycerols, fatty acids, and monoacylglycerols, steroids, sterols, straight-chain and ring-containing alcohols. Various lipids include vitamins E and K, squalene, certain wax lipids, and carotenoids (Senbagalakshmi *et al*., 2019). Due to their high caloric content, as well as the essential fatty acids and fat-soluble vitamins present in natural food fat, lipids are significant nutritional components. The body is unable to manufacture linoleic acid, an important fatty acid. The maintenance of the functionality and integrity of membrane structure, prostaglandin synthesis fat metabolism all depend on linoleic acid. Scaly dermatitis is a very common sign of an essential fatty acid deficiency. Adipose tissue, where fat is deposited, serves as a thermal insulator in subcutaneous tissues and surrounding particular organs. Depolarization

waves can quickly travel along myelinated nerves thanks to the electrical insulating properties of nonpolar lipids. Lipoproteins are crucial biological components that exist in both the mitochondria and the cell membrane. They also act as a route of lipid distribution in the body system (Sirwi and Hussain, 2018). When eating fatty meals, there is a propensity to feel full afterward since fats in food make food more palatable.

Dietary fats have shown to have a significant impact on the incidence of obesity, cancer, and coronary heart disease. Chronic disease risk is enhanced by high cholesterol or Low Density Lipoprotein (LDL) levels. The risk of coronary heart disease has, however, been associated to high levels of High Density Lipoprotein (HDL) cholesterol (Lee *et al*., 2017). Consuming monounsaturated fats lowers HDL cholesterol while maintaining or lowering total cholesterol and LDL cholesterol. Saturated fat consumption is highly associated with increased levels of LDL and total plasma cholesterol (Rock *et al*., 2017). LDL and HDL cholesterol have been found to decrease when n-6 fatty acid-containing lipids (linoleic acid) are consumed. Dietary n-3 polyunsaturated fatty acids (linolenic acid) significantly lower the risk of cardiovascular mortality, suppress cardiac arrhythmias, lower blood pressure, reduce serum triacylglycerols, and decrease the potential for thrombosis (Sokoła-Wysoczańska *et al*., 2018).

2.8.4 Dietary Fiber

Non-starchy polysaccharide and lignin are two terms used to describe dietary fibers. Dietary fibers are those parts of food that human digestive enzymes are unable to break down. Soluble fibers and insoluble fibers are the two main categories of dietary fibers.

Pectins and gums are examples of soluble fibers, whereas cellulose, hemicellulose, and lignin are examples of insoluble fibers. In the digestive tract, soluble fibers dissolve in water to form a gel that slows down the passage of food. Fibers that are insoluble in water are insoluble. They cause the intestines' rhythmic muscle contractions, known as peristalsis, which move the digestive system's contents along (Le *et al*., 2021). High-fiber diets have a number of health advantages. Dietary fibers work to lower the likelihood of gastrointestinal issues like diarrhea and constipation. Lignins have properties that improve bulk and absorb organic materials like cholesterol to lower plasma cholesterol levels. Ingesting fibers together with foods high in carbohydrates considerably reduces the increase in blood sugar and insulin levels because gums and pectins commonly known as mucilaginous fibers, create viscous gels inside the intestine and stomach and reduce the pace of gastric secretion. The majority of people's water-soluble fiber intake also contributes to lower serum cholesterol (Singh *et al*., 2017b).

2.9 Antinutritional Factors (ANFs) in Plant Foods

Antinutritional factors, also known as anti-nutrients (Phytochemicals), are substances present in natural food products which were synthesized during normal metablic processes involving many mechanisms (such as the inactivation of some nutrients, a slowing down of the digestive process, or a metabolic utilization of feed) that have a counter effect to optimal nutrition (Thakur *et al*., 2019).

Antinutrients are sometimes referred to as substances that plants have developed for their own protection. Plants create them to protect themselves from fungi, insects, and predators as well as to provide a defense mechanism for the plant. If ingested in the right levels, several of these antinutrients have been proved to be harmful to health or to be clearly beneficial to human health (Sinha and Khare, 2017).

Antinutritional factors are present in various food products, and in different extend depending on the food-type, processed method, the chemicals used for cultivation, food preservation and storage (Thakur and Kumar, 2017; Uzoukwu *et al*., 2020). In general, antinutrients can be divided into 2 main categories: the heat-stable and the heat-labile group (Kumar *et al*., 2011; Zhou *et al*., 2018). Condensed tannins, phytic acid, saponins and alkaloids are examples of heat-stable antinutrients that can withstand and remain stable at high temperatures; in contrast, toxic amino acids, lectins, protease inhibitors, and cyanogenic glycosides are examples of heat-labile antinutrients that are susceptible to ambient temperature and lose their potency at high temperatures (Thakur *et al*., 2019).

Antinutrients, often referred to as secondary metabolites, are good sources of minerals and help to meet the RDA for key elements. They can be used in nutrition and as pharmacologically active agents (Ehsen *et al*., 2016). Nikmaram *et al*. (2017); Melini and Melini (2021) and John *et al*. (2017) have investigated and reported the anti-nutritional properties of seeds. These anti-nutrients are prevalent in almost all seeds. The following explains all of these antinutritional elements, their content in plants, and how they affect digestibility.

2.9.1 Cynogens

Cyanogens are the cyanide-containing glycosides of a sugar, typically glucose, and an aglycone. Phytoanticipins are a category that includes cyanogenic glucosides. Their general purpose in plants depends on activation by b-glucosidases to produce hazardous volatile Hydrogen cyanide, aldehyde or ketone to repel pathogen and stop herbivore attack (Sinha and Khare, 2017). Cyanogenic glycosides, also known as cyanoglycosides,

make up about 90 % of the wider class of plant toxins known as cyanogens. The primary feature of these poisons is cyanogenesis, which results in the production of free HCN and is linked to cyanohydrins which have already undergone glycosylation (the attaching of sugars) to stabilize them and produce cyanogenic glycosides (Gulhane and Jadhao, 2019).

The edible parts of plants are known to contain at least 25 cyanogenic glycosides (Bolarinwa *et al*., 2016). Chemically speaking, linamarin is comparable to glucose but has cyanide (CN ion) attached. Cyanide increases the rate of glycolysis by activating glycogenolysis, transferring glucose to the hexose monophosphate shunt and inhibiting the tricarboxylic acid (TCA) cycle (Mosobalaje *et al*., 2019).

The cyanogenic glucosides lotaustralin and linamarin are the principal antinutritional components of cassava. More than 2500 distinct plant species, such as white clover, linseed, various sorghums, and cassava (*Manihot esculenta*), have been reported to possess cyanogenic glucosides (*Trifolium repens*).

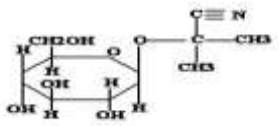


Figure 2.8: Molecular Structure of Linamarin

Source: Ekop (2020)

Several plants, including the roseroot (*Rhodiola rosea*), white clover (*Trifolium repens*) and lima bean (*Phaseolus lunatus*) contain trace amounts of lotaustralin, a cyanogenic glucoside. The only structural difference between lotaustralin and linamarin, another glucoside discovered in these plants, is the inclusion of an additional methyl group. The enzyme linamarase has the ability to hydrolyze both lotaustralin and linamarin, resulting in the formation of glucose and a deadly chemical called hydrogen cyanide.

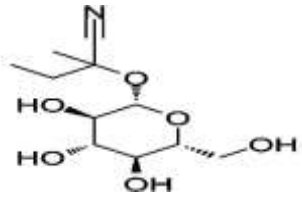


Figure 2.9: Molecular Structure of Lotaustralin

Source: Ekop (2020)

Both lotaustralin and linamarin are produced in the leaf and then transported to other sections of the plant, primarily the roots, primarily through the phloem vessels. They coexist with the enzymes -glucosidase or linamarase in various compartments, and neither the glucosides nor the enzymes are toxic on their own (Ekop, 2020). To put it another way, healthy plants do not contain free cyanohydrins or hydrocyanic acid, but they may do so in response to environmental conditions like drought or processing-related cellular damage that slow or stop normal growth, or when people or animals consume cyanogenic plants and the glucoside is hydrolyzed by the microbial flora in their intestines (Cressey and Reeve, 2019). Glucosides are hydrolyzed to release hydrocyanic acid glucose and acetone cyanohydrin, the former of which is toxic to both humans and animals (Schrenk *et al*., 2019).

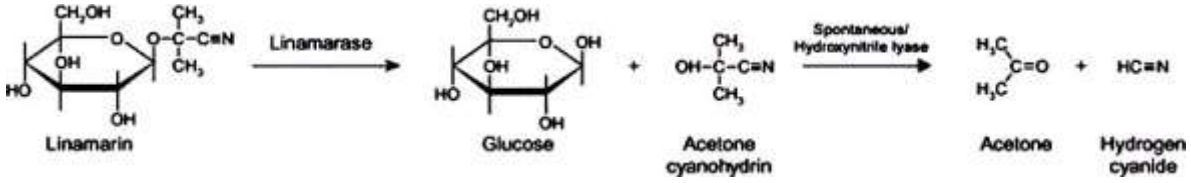


Figure 2.10: Formation of HCN from Linamarin

Source: Schrenk *et al*. (2019)

2.9.1.1 Toxicity and metabolism of cyanide

Compounds in seeds that contain cyanide are typically harmful instead of useful. However, many cyanogenic seeds have also been consumed as delicacies for a long period

of time because of their high nutritional value. In several cases, developing lowcyanogenic plant species has also considerably improved food safety. Plants that are naturally high in cyanogen are now available in forms that are remarkably low in cyanogen. Cyanogenic seeds used mostly for human consumption or the items made from them often have cyanide levels below critical thresholds when properly handled (Abraham *et al*., 2016; De Girolamo *et al*., 2022).

Hydrogen cyanide can be effectively digested by animals at low (sub-lethal) amounts. The primary mechanism by which the body defends itself against the harmful effects of cyanide is the enzyme mitochondrial sulfur transferase's conversion of cyanide to thiocyanate. The dietary sulfur amino acids methionine and cysteine are the main sources of the sulfur donors needed for the enzymatic detoxification. Rhodanese is a multifunctional enzyme that catalyzes the production of sulfite and thiocyanate and from thiosulfate and cyanide or some other appropriate sulfur donors in vitro (Figure 2.11) (Onojah and Odin, 2015; Bonanno, 2020).

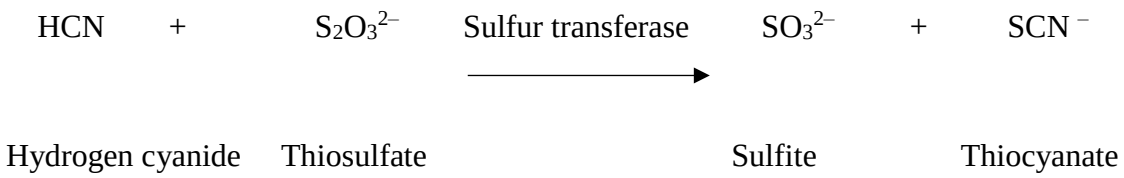


Figure 2.11: Processes of Cyanide

Source: Onojah and Odin (2015)

Sulfur transferase is a mitochondrial enzyme that converts cyanide to thiocyanate. Thiosulfate and cyanide interact with an active disulfide group in the enzyme. Sulfur donors, which are needed for this detoxification, are provided by the amino acids in the diet that contain sulfur. Urine eliminates thiocyanate from the body.

2.9.1.2 Biochemical effect and health impact of cyanide

Although the majority of cyanogenic foods are safe in industrialized nations, humans can effectively digest lower doses of hydrogen cyanide. When cyanogenic glucoside is hydrolyzed, poisonous hydrocyanic acid is produced (HCN). The cyanide ions affect a number of enzyme systems, slow growth by interfering with the utilization of related nutrients and a number of critical amino acids. Additionally, they produce acute toxicity, neuropathy, central nervous system abnormalities, cardiac arrest, respiratory failure, and death (Umar, 2019).

The clinical signs of cyanogenic glycoside poisoning in humans are similar to those of cyanide poisonings, and the severity of poisoning is inversely correlated with the level of cyanogenic chemicals that are active. Abdominal pain, Vomiting, diaphoresis, weakness and dyspnea are the first symptoms of disorientation, convulsions, stupor, metabolic acidosis and hypotension. These symptoms are then followed by deeper and faster breathing, irregular, a faster, salivation and foaming at the mouth, weaker pulse, bright red mucous membranes, muscular spasms and dilated pupils. High dosages of cyanogenic glycosides cause cardiac collapse, coma and respiratory failure (Cressey and Reeve, 2019; Mosayyebi *et al*., 2020).

It is important to keep in mind that the HCN generated from precursors containing cyanide is a potent respiration toxin and therefore the World Health Organisation (WHO) recommendation of cyanogen-containing food plants (10 mg/kg) should be followed. The enzyme cytochrome oxidase is inhibited by hydrocyanic acid, which prevents the oxidative generation of energy (Analin *et al*., 2020). Under conditions of salinity stress, the alternative oxidase and cytochrome oxidase pathways of the mitochondrial electron transport are crucial for pea plants' ability to perform photosynthetically, and they also

prevent oxygen from binding to hemoglobin (Shivaraj *et al*., 2020). By attaching to the Fe^{3+}/Fe^{2+} present in cytochrome oxidase, hydrogen cyanide renders the enzyme inactive in cellular mitochondria. As a result, the tissues' ability to use oxygen declines. Cyanide results in a rise in the levels of blood glucose and lactic acid as well as a change from aerobic to anaerobic metabolism and a drop in the ATP/ADP rate.

Cyanide increases the process of glycolysis by activation of glycogenolysis, diverting glucose to the hexose monophosphate shunt, and inhibiting the tricarboxylic acid (TCA) cycle. All cells will experience a decrease in energy availability due to hydrogen cyanide, but the respiratory system and heart will feel the effects the fastest. Dietary cyanogens present in incorrectly processed cassava products have all been linked to protein malnutrition, tropical ataxic neuropathy (TAN) and diabetic mellitus (RivadeneyraDomínguez and Rodríguez-Landa, 2020). They have also been proven to produce chronic cyanide toxicity. Konzo, a paralytic disease, mainly affects children over 3years and those women who are fertile and eat a diet high in bitter cassava roots, in contrast to TAN, which occasionally occurs in individuals below 10years (Netto *et al*., 2016). It is now well established that iodine deficiency disorders (IDDs), primarily manifested as goiter and cretinism, can be made worse by the thiocyanate load brought on by dietary cyanide exposure from cassava (Sánchez-Pérez *et al*., 2008).

2.9.2 Alkaloids

The chemical compounds known as alkaloids, which are often present in plants as salts of plant acids like oxalic, malic, tartaric, or citric acid, are among the most prevalent categories of chemical compounds created by plants. There are several bitter plant substances called alkaloids in nature, and they frequently have medicinal effects. Alkaloids are frequently basic nitrogen-containing substances that can form salts with

acids, and they serve as secondary plant metabolites in the majority of cases (Leitão *et al*., 2021). At least 40 % of the plant families have roots, seeds, leaves, or bark that have been found to contain alkaloids. The Leguminosae, Liliaceae, Papaveraceae, Compositae, Amaryllidaceae, and Solanaceae families all contain a lot of alkaloids. Both plants and common foods contain alkaloids. A type of alkaloids that is prevalent in the kingdom of plants are the pyrrolizidine alkaloids, solanine and tomatine are common examples of these alkaloids (Akinboye *et al*., 2023). Tomatine is an alkaloid found in tomatoes, whereas solanine is an alkaloid found in potatoes in lesser concentrations. Glycoalkaloids are often better absorbed at alkaline pH settings because their attachment to sterols in cell membranes causes further breakage. Alkaloids include well-known substances including nicotine (from tobacco), cocaine (from coca leaves), caffeine, quinine (from cinchona bark), morphine (from dried opium poppy latex), strychnine and solanine (from immature potatoes and potato sprouts) (Sinha and Khare, 2017).

About 15 to 20 % of all vascular plants contain alkaloids, which are tiny chemical compounds. They typically consist of a number of side-chained carbon rings with one or more nitrogen atoms in place of carbon atoms. They are created by plants from amino acids. When amino acids are decarboxylated, amines are produced, which then react with amine oxides to produce aldehydes. Aldehyde and amine group condensation of the Mannich type results in the formation of the distinctive heterocyclic ring in alkaloids (Thakur *et al*., 2019).

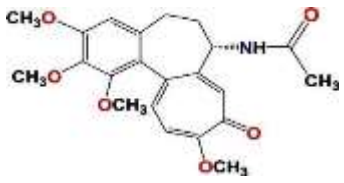


Figure 2.12: Chemical Structure of Alkaloids

Source: Thakur *et al*. (2019)

2.9.2.1 Health Implication of Alkaloids

Alkaloids are regarded as anti-nutrients because of how they affect the neurological system, which can interfere with or unnaturally promote electrochemical transmission. High tropane alkaloids, for instance, can cause a fast heartbeat, paralysis, and, in the worst case, death. Tryptamine alkaloids can induce stumbling and death in excessive doses. Alkaloids have undeniable physiological impacts on people (Klotz, 2015). Gastrointestinal and neurological issues are frequently brought on by alkaloids (Kwakye *et al*., 2018). According to Hennessy *et al*. (2020), the glycoalkaloids solanine and chaconine, which are present in potatoes and Solanum spp are haemolytically active and poisonous to both fungus and people. In particular at doses greater than 20 mg/100 g sample, neurological problems and gastrointestinal disturbances are some toxicological symptoms of potato glycoalkaloids. Some plant alkaloids have been associated to infertility, while coumarins, which are fodder ingredients, have been connected to bleeding sickness in cattle fed rotten or putrid sweet clover (Alafid *et al*., 2019). Important pharmacological effects such analgesia, blood pressure decrease, tumor cell death, respiration stimulation and circulation stimulation are mediated by a low dose of alkaloids (Mohammed *et al*., 2019).

2.9.3 Saponins

Many plant species, including peanut, lupin, oil seeds, and others, have water-soluble plant components called saponins that, even in small concentrations, can produce soapy foam (Samtiya *et al*., 2020). They are glycosides with a non-sugar aglycone component that resembles sapogenin. The bitter taste and red blood cell hemolysis properties of saponins make them stand out. Based on the chemical makeup of the sapogenin, they are separated into two main groups: triterpenoid saponins and steroidal saponins.

All portions of plants contain saponins, though the concentration varies depending on the type and stage of growth. Saponins are present across the whole plant kingdom. They are present in variety of pulses and oil seeds, including alfalfa, ginseng root, kidney bean, chickpea, soybean, groundnut, lupin, sunflower, sugar beets, peanuts, asparagus, broccoli, potatoes, and sugar beets (Kirkensgaard, 2021).

The following can be listed as the general traits of saponins: they have a bitter taste and froth when they are exposed to different aqueous solutions.

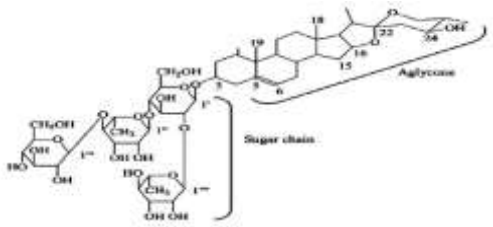


Figure 2.13: Chemical Structure of Saponins

Source: Samtiya *et al*. (2020)

2.9.3.1 Health implication of saponins

Red blood cells may hemolyze because of the foam that saponin creates. They have a very harmful effect on cold-blooded species because they lower the surface tension of their blood. Food plants become astringent and bitter when saponin levels are high. The primary barrier to saponin's use is its bitter taste. Saponins was recognized as anti-nutrient components because of their detrimental consequences, such as growth retardation and throat irritation (Oleszek and Oleszek, 2020). Additionally, it was discovered that saponins inhibit a number of digestive enzymes, including trypsin and chymotrypsin, which has an impact on the digestibility of proteins (Samtiya *et al*., 2020). The pharmacological and therapeutic effects of saponins are the result of their complex structural makeup. Because of the advantages saponins provide for humans, they are

generating a lot of interest. Recent research reveals that saponins have anticancer, immunostimulatory, and hypocholesterolemic effects (Chatterjee *et al*., 2018). Additionally, they lower the risk of cardiac problems in people who eat a diet high in legumes that contain saponins.

The importance of saponins for health is indicated by the reality that they could bind to cholesterol and limit bioavailability as a result, and that they are present in legumes. Human diets should include foods high in saponins to lower the risk of heart disease, manage plasma cholesterol, avoid peptic ulcers, and osteoporosis (Thakur *et al*., 2019). Red blood cells can be damaged by saponins, which can also cause diarrhea and vomiting. By interfering with intraluminal physicochemical processes, saponins reduce the bioavailability of several nutrients, such as cholesterol and glucose, at the intestinal level. This leads to claims that it has hypocholesterolemic effects (Kumari *et al*., 2017; Del Hierro *et al*., 2021). Additionally, it has been shown that saponins have an anti-spermal effect on human spermatozoa. They considerably reduce the human sperms' ability to synthesize acrosine, and this spermicidal effect is due to severe damage to the spermal plasma membrane (Aremu *et al*., 2016).

2.9.4 Phytates

In soil, plants, animals, and other living things, phytate (Inositol hexakisphosphate, or InsP6) is a salt form of phytic acid. In the seed of many of our grain legumes, inositols with 4, 5, or 6 phosphate groups are frequent, with concentrations exceeding 10 % dry matter. Phytates can be easily separated by milling since they are found in the germ of maize and the aleurone or bran layer of monocotyledons like wheat and rice. However, phytates are frequently extracted from or concentrated with the protein portion of dicotyledon seeds like legumes, nuts, and oilseeds. They are found tightly connected with proteins in these foods. They could be thought of as phosphate and mineral nutrient stores,

vital for plant nutrition and particularly sensitive when germinating (Veer *et al*., 2021). Phytates can lead to mineral ions deficit in both humans and animals because they include, complex zinc, iron, calcium ions and magnesium in the digestive tract. Once more, these substances appear to function as phosphate and mineral stores in addition to defense. Enzymes that hydrolyze phytates can be added to foods to reduce their phytate content (Sinha and Khare, 2017). According to some studies, phytates account for between 50 % and 80 % of phosphorous present in seed and because foods from plant source contain more phytic acids than foods from animal source, vegan diets are more prevalent in underdeveloped countries, which raises dietary phytic acid intake (Bhadouria *et al*., 2017; Raboy *et al*., 2020).

Many of the major legumes and oilseeds contain significant amounts of phytic acid. Soybean, rapeseed, and cotton seed are examples. Other minerals appear to chelate to phytic acid as it accumulates in storage sites in seeds, creating the complex salt phytate. 1 to 2 % phytic acid is said to be present in whole soybeans. Some natural enzymes are active during seed germination and break down phytic acid (Pakfetrat *et al*., 2019). The majority of the phosphorus included in phytic acid is mainly inaccessible to animals because monogastric animals lack the phytase enzyme in their digestive tracts. Phytic acid in hens is significantly inversely linked with the availability of calcium, magnesium, phosphorus, and zinc in diets such as palm kernel seed rapeseed, soybean meals and cotton seed (Thakur *et al*., 2019).

2.9.4.1 Health implication of phytates

Mineral-phytic acid and Protein complexes are formed by phytic acid, which is a strong chelator, and as a result, the bioavailability of these nutrients is decreased (Verma *et al*., 2017). It has been reported that metal ions like molybdenum, zinc, magnesium, iron, copper and calcium chelate in phytic acid, forming insoluble complexes that are difficult

to absorb from the gastrointestinal tract. Pepsin, tyrosinase, trypsin, amylase and lipase activity are all inhibited by phytic acid in the gastrointestinal tract. The reduction in zinc bioavailability caused by phytic acid is, the most significant impact of this compound on human nutrition (Gibson *et al*., 2018).

Phytic acid significantly affects children, pregnant women and nursing women because when cereal-based foods are consumed in large amount, it decreases the bioavailability of vital minerals and turns them into insoluble molecules, where low amount is digested and absorbed inside the small intestine (Al Hasan *et al*., 2016). An earlier study found that phytic acid impairs the ability of enzymes required for protein breakdown inside the small intestine and stomach (Pei *et al*., 2019).

Dietary phytate may also be beneficial for diabetic patients' health since it decreases blood glucose responses by slowing stomach emptying and reducing the pace of starch breakdown. Phytate has also been demonstrated to control the release of insulin (Omoruyi *et al*., 2020). According to popular belief, phytate prevents cardiac ailments by lowering blood clotting, cholesterol, and triglycerides. It may also stop the formation of kidney stones, according to some research. It serves as a complexing agent to get rid of heavy metal ions' remnants (Thakur *et al*., 2019).

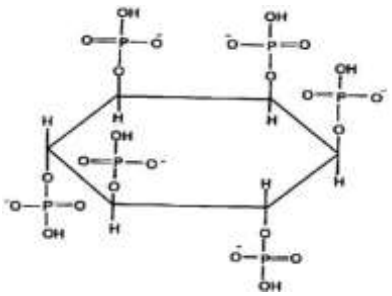


Figure 2.14: Chemical Structure of Phytate

Source: Gibson *et al*. (2018)

2.9.5 Oxalates

Oxalate is a dicarboxylic acid that has a 1:1 molar stoichiometry with calcium to generate an insoluble calcium salt. In addition to several plants, fruits, and virtually all nuts and seeds, oxalates are chemical compounds found naturally in our body systems. Mammals are unable to access these minerals due to the interaction of oxalic acid with Mg^{2+}, Ca^{2+}, and Fe^{2+}. It can also create salts that dissolve in water with NH_4^+, Na^+ and K^+. Zn^{2+}, however, seems to be mostly unaffected. Oxalic acid and its salts are byproducts of metabolism in many plant tissues. Oxalate binds calcium and other minerals, yet oxalic acid is a typical end product of mammalian metabolism, hence consuming these plants may have negative effects. Oxalic acid can form kidney stones if it is consumed in excess and then excreted in the urine (Bartges, 2016).

Although oxalate is present in many plants, it only has a small number of nutritionally significant species. The families listed below have the greatest oxalate concentrations: Goosefoot family members include *Beta* (beet and beet root), *Spinacia* (spinach) and *Oarch* (atriplex); *Amaranthus araceae* (amaranth) which includes *Xanthosoma* (caladium) and *Colocasia* (taro); and wood sorel family members include Oxalis (*Sorrel yam*). Oxalate is distributed unevenly inside plants as well. The amount of oxalate is greatest in leaves, then in seeds, and least in steams. When designing low-oxalate diets, plants can be regarded as oxalate-free because they contain more oxalic acid than meat does (Savage and Klunklin, 2018; Soyingbe *et al*., 2020).

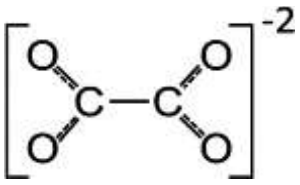


Figure 2.15: Molecular Structure of Oxalate

Source: Savage and Klunklin (2018)

2.9.5.1 Health implications of oxalate

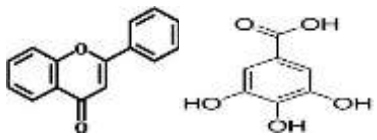
Oxalates bind to calcium and crystallize when they come into contact with injured tissues, irritating and hurting the tissues. This, in turn, either fuels or exacerbates inflammation. When the crystals implant themselves where they obstruct the passage of other material, the discomfort can be more severe (such as in your digestive tract). Furthermore, a "leaky" or porous stomach permits a significant amount of oxalates to enter the body. Numerous health issues, such as hypothyroidism, vulvodynia, autism, kidney stones and fibromyalgia, to name a few, are linked to this overabundance. Oxalic acid and its salts, particularly calcium oxalate, can negatively impact human nutrition and health, for instance, by lowering calcium absorption and encouraging the formation of kidney stones (Brzezicha-Cirocka *et al*., 2016; Sinha and Khare, 2017).

Oxalates from human metabolism and dietary sources are both eliminated in urine. However, not all oxalates are soluble, and high levels of urinary oxalate can crystallize and form stones. Calcium from the body and from other sources in the diet can be bound by soluble oxalate in the diet (Bong *et al*., 2017). Calcium phosphate/calcium oxalate and calcium salts are present in about 85 % of all kidney stones. High oxalate intake affects people in ways other than the production of oxalate stones. Small amounts of oxalate in the body can result in headaches, cramps, and muscle pain and twitching. Higher doses may result in indications of heart failure, an irregular and weak heartbeat, and reduction in blood pressure. Large dosages of oxalate can quickly induce a shock-like state, which can result in convulsions, a coma, and even death. The mean reported LD_{50} is just 5 g (approximately 70 mg/kg), but the average LD_{50} for an adult is between 15 and 30 g (Karami *et al*., 2021).

2.9.6 Tannins

Tannin, a polyphenol found in the seed coats of almost all legumes, is an anti-nutritional factor (Rahate *et al*., 2021). Gallotannins and ellagitannins are examples of hydrolyzable tannins, while proanthocyanidins are examples of condensed tannins (de Camargo and da Silva Lima, 2019). The hydrolyzable tannins are flavanol polymers known as condensed tannins that produce anthocyanidins after heating in an acidic solution. They are classified as non-flavonoids and have gallic acid serve as their precursor.

The digesting process in ruminants easily breaks down tannins of the hydrolysable type. There are numerous chemicals in the breakdown products, some of which might be toxic (Samtiya *et al*., 2020). Generally, condensed tannins make up the majority of legumecontaining fodder and some seeds. Previous research has shown that whereas goats are not sensitive to these tannins, sheep and cattle are (Smeriglio *et al*., 2017; Bhattarai *et al*., 2016). The proanthocyanidin unit, which is where the condensed tannins are located, is what gives the seed its color. Enzymatic hydrolysis of condensed tannins produces sugar and phenol carboxylic acid. Three benzene rings make up the structure of anthocyanin, a tannin derivative and a distinctive feature of polyphenols. It has been discovered that proteins with a high proline concentration (25 – 45 %) have a high affinity for tannins (Soares *et al*., 2018). Figure 2.16 shows the structure of hydrolysable and condensed tannins (Lamy *et al*., 2011).



Gallic Flavone

Figure 2.16: Structures of Hydrolysable (Gallic) and Condensed (flavone) Tannins

Source: Lamy *et al*. (2011)

2.9.6.1 Health implication of tannins

The hydroxyl group of tannins can form reversible and irreversible tannin-protein complexes with the carbonyl group of proteins, this will decrease the digestibility of proteins and then prevent access to essential amino acids (Kurzbaum *et al*., 2019). The bulk of proteins that interact with tannins are large, hydrophobic, have flexible and open structures that are proline-rich (Shahidi and Dissanayaka, 2023). When consumed, tannins interact with proteins to form complexes that block a number of digestive enzymes and decrease the protein's ability to be digested (Joye 2019; Samtiya *et al*., 2020).

2.9.7 Flavonoids

Flavonoids are a group of many low molecular weight secondary plant phenolics, distinguished by the flavan nucleus. More than 4,000 flavonoids have been identified currently; they are abundant in plant leaves, bark, seeds, and flowers and have been shown in numerous models to be efficient antioxidants. These substances offer plants protection from ultraviolet radiation, diseases, and herbivores (Gulcin, 2020).

The subgroups of flavonoids are based on the level of oxidation and unsaturation and of both the C ring and the carbon of the C ring which links the B ring (Figure 2.7). For instance, In the flavonoids called isoflavones and neoflavonoids, a B ring is attached to the third and fourth position of the C ring respectively. Furthermore, there are several subgroups of compounds in which the B ring is linked to the C ring to position 2, the names given to these compounds depend on the structural properties of the C ring. Anthocyanins, flavones, flavanones, flavonols, flavanonols and chalcones are some of these subclasses (Patil *et al*., 2020).

The post-ischemic heart damage in rats was reported to be lessened by flavonoids in the diet (Ahmed *et al*., 2020). The human diet may have a protective effect, according to certain significant prospective research. Mendonça *et al*. (2019) have shown a lower risk (38 %) of occurrence of coronary heart disease in postmenopausal women with high flavonoid, also, older men with high flavonoid consumption have lower risk of death from coronary heart disease and decreased prevalence of myocardial infarction (Mayr *et al*., 2018). Flavonoids may be protective in biological system because of their capacity to chelate metal catalysts, activate antioxidant enzymes, inhibit oxidases, and decrease alpha-tocopherol radicals (Neunert *et al.*, 2015; Pisoschi *et al*., 2021).

In nature, flavonoids serve a variety of roles, including: attracting pollinators and seed dispersers by coloring flowers, fruits, and seeds; protecting plants from ultraviolet light; defending plants against pathogenic microorganisms; promoting plant reproductive function; pollen growth and serving as chemical messengers in plant-microbe environment interactions (; Chagas *et al*., 2018; Nunes *et al*., 2018; Paauw *et al*., 2019).

Flavonoids are essential part of the human diet since they play significant role to the appearance and taste of fruits, vegetables, nuts, and seeds (Panche *et al*., 2016). Soybeans (isoflavones), tea, citrus (flavanones), apple, celery (flavones), cocoa, berries and onions (flavonols) are just a few examples of foods that are rich in flavonoids (anthocyanins) (Singh *et al*., 2017a; Guven *et al*., 2019).

Colored anthocyanins in flavonoids have made them a well-liked research subject in the past. particularly, the discovery of transposable elements by McClintock's affected maize pigment biosynthesis genes and Gregor Mendel's work on inheritance for genes which are important for pea seed coat color have found use for the attractive anthocyanin colors in genetic investigations (Su *et al*., 2020; Zhang *et al*., 2020; Jo *et al*., 2021).

2.9.7.1 The flavonoid biosynthetic pathway

The flavonoid pathway constitutes two types of genes, structural and regulatory genes. The structural genes which can be auto regulated by flavonoids are involved in the synthesis of enzymes of enzymes which are directly involved in flavonoid biosynthesis.

The bulk of flavonoids are produced from the precursors p-coumaroyl-CoA and malonylCoA and, these precursors are metabolites of carbohydrate metabolism and phenylpropanoid pathway respectively (Milke *et al*., 2019). Chalcone synthase (CHS) initiates the biosynthesis of flavonoids, this enzyme synthesizes the yellow chalcone. Chalcones are not the required end products in flavonoid biosynthesis for most plants, instead, the process advances through a number of enzymatic stages, synthesizing other groups of flavonoids, like dihydroflavonols and flavanones, then on to anthocyanins, which are the primary water-soluble colors in flowers and fruits. More groups of flavonoids, such as isoflavones, aurones, flavonols, pro-anthocyanidins and flavones are created from intermediates in the synthesis of anthocyanins due to side branching of flavonoid biosynthetic pathway.

2.9.7.2 Classification and chemical structure of flavonoid

The classification of flavonoids, which are benzo-pyrone derivatives with phenolic and pyrane rings, is based on the substitutions that they undergo (Figure 2.8). Different dietary flavonoids have different arrangements for the methoxy, hydroxyl, and glycosidic side groups as well as different combinations of the A and B rings. The hydroxyl groups are added to, sulfated, methylated, and glucuronidated throughout metabolism. Polymers and 3-O-glycosides are the two main types of flavonoids present in food (Li *et al*., 2020). There are many forms of higher structure, and dietary flavonoid consumption is largely made up of polymers (Wojtunik-Kulesza, 2020). Enzyme-aided oxidation transforms

flavanols into tannins and certain complex compounds when green tea leaves (*Camellia sinensis*) are fermented into black tea (Jiang *et al*., 2019). Flavanol units make up condensed tannins, also known as proanthocyanidins. The procyanidins are those substances that are most pertinent to the human diet; they are made up of (epicatechin and catechin monomers). The 436 and 438-linked procyanidin dimers, trimers, and oligomers are present in, seeds, grape red wines, cocoa and apples (Díaz-Mula *et al*., 2019).

Proanthocyanidins have the potential to have large molecular weights and contain up to 17 flavanol units (Quijada-Morin *et al*., 2015; Suo *et al*., 2019). Gallotannins, also referred to as hydrolyzable tannins, are gallic acid esters. The chelating and radical scavenging properties of monomeric catechins and tannins of green tea are in part because of the galloyl moieties present in these compounds (Ricci *et al*., 2018; Sen *et al*., 2020).

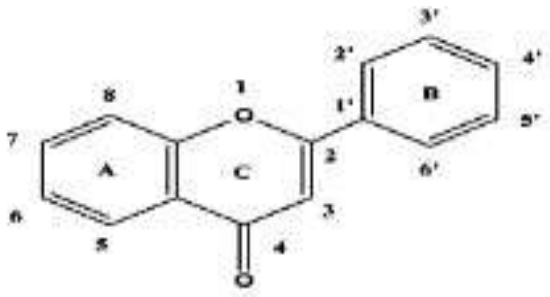


Figure 2.17: Nuclear Structure of Flavonoids

Source: Wojtunik-Kulesza (2020)

Dietary flavonoids come in a variety of forms and differ in terms of hydroxylation patterns, aromatic ring conjugation, glycosidic moieties, and methoxy groups. In black tea, red wine and grapes, polymerization of this nuclear structure will produce tannins and other complex species.

2.9.7.3 Health implication of flavonoids

The majority of flavonoids' beneficial health effects are a result of their chelating and antioxidant properties. This leads to numerous health benefits of flavonoids such as distinct cardioprotective benefits by preventing the oxidation of low-density lipoprotein, anti-inflammatory effects and the protection of cells from oxidative stress that can result in diseases (Luo *et al*., 2017; Ullah *et al*., 2020). These dietary antioxidants can slow the onset of diseases like diabetes cancer, alzheimer's, dementia cardiovascular diseases and other cognitive disorders. Regular consumption of foods containing flavonoids may lower the risk of many chronic diseases such as neurodegenerative diseases, atherosclerosis, numerous types of cancer and type 2 diabetes, according to evidence from observational studies (Atrahimovich *et al*., 2021).

There are numerous ways that flavonoids might benefit the body. For instance, incorporating foods rich in flavonoids into your diet may be a useful strategy for managing high blood pressure. There are at least five subtypes of flavonoids that have been shown to decrease high blood pressure (Garcia-Yu *et al*., 2018). Furthermore, the flavonoids present in tea, coffee, and soy may reduce the chance of suffering a heart attack or stroke. According to Parmenter *et al*. (2020), adults who included more flavonoids in their diets were less likely to have a cardiovascular incident. To demonstrate the cardiovascular advantages of flavonoids, more research is necessary (Parmenter *et al*., 2020).

2.10 Reduction or Elimination of Antinutrients in Foods

Antinutrients have the ability to reduce the amount of certain minerals and nutrients in the body, but they also have the potential to be poisonous in excessive doses (Sango *et al*., 2016). These factors make it important to reduce foods' antinutritional content. They

can sometimes be almost entirely eliminated**.** To decrease these antinutrients in foods, various traditional and modern processing methods like milling, soaking, roasting debranning, cooking, fermentation and germination have been employed (Samtiya *et al*., 2020). Various techniques employed to reduce the levels of cyanide, phytate, saponins, tannin, and other contaminants in foods are described below:

2.10.1 Soaking

Soaking is a desirable strategy for removing foods' antinutrient content, given that it shortens the cooking process. The majority of antinutrients simply dissolve when meals are soaked since they are water-soluble (Nguyen *et al*., 2018). Since most antinutrients are water-soluble, they simply disintegrate when meals are soaked (Nguyen *et al*., 2018). Soaking also encourages the release of natural phytases and other enzymes in plant-based foods including some nuts, cereals, and almonds. It is necessary to frequently immerse grains, edible seeds, and nuts in water for a while in order for them to germinate and lower the amount of enzyme inhibitors and other anti-nutritional factors, this would increase their nutritional content and digestibility (Kumari, 2018). Soaking is commonly required for fermentation, which can be used to reduce the level of several anti-nutrients in foods (Petroski and Minich, 2020).

According to Ojo *et al*. (2018), phytic acid in Mucana flagellipes decreased by 58.4 and 74.9 % after 6 hours and 24 hours of soaking, respectively, at room temperature. Phytase activity increased, lowering the amount of phytate in the grains because of the soaking. Due to the water-soluble vitamins and minerals that are leached from grains and legumes during soaking and fermentation, phytochemicals are diminished (Oliviero and Fogliano, 2016). *In vitro* solubility of minerals like zinc and iron could be increased by 2 to 23 % by exogenous or endogenous phytase enzymes during soaking (Vashishth *et al*., 2017).

In fact, the soaking beans and grains is an extremely effective way to boost the quantity of protein and minerals available while simultaneously lowering the level of phytic acid (Shi *et al*., 2018). Samtiya *et al*. (2020) revealed that long-term soaking of chickpeas (two to twelve hours) decreased their phytic acid content by 47.45 - 55.71 %. The reduction of cooking time has traditionally been the aim of this procedure. By promoting antinutrient transport and disintegration into the aqueous phase, this procedure hydrates the seeds. The body absorbs nutrients better and has better digestion as a result of soaking. The amount of antinutrient factors depends on how long the food is soaked. 8 to 12 hours is the typical soaking time at room temperature (Diouf *et al*., 2019). Studies have supported or even surpassed this phytate loss. In fact, a decrease in phytate level of 22.4 and 23.7 % was achieved on two kinds of cowpea following 24 hours of soaking. It is being tested to use sodium bicarbonate as another dipping solution. Antinutritional factor levels can be significantly reduced by adding sodium bicarbonate ($NaHCO_3$) to the water used for soaking. In fact, three different red beans species reduced in total alphagalactoside (raffinose and stachyose) by 41, 45 and 40 % after 12 hours of soaking them in sodium bicarbonate (Bharadwaj *et al*., 2021). These findings indicate that the content of reduced antinutritional factors varies depending on the soaking solution used. In comparison to soaking in plain water, the decrease was increased by the adding sodium bicarbonate. The seed's tenderness enabled the quick dissolving of the components in the soaking solution, which accounts for the fast reduction seen after adding sodium bicarbonate. These studies demonstrate that soaking is an effective method for lowering antinutritional components in legumes like cowpea. So, there are various theories as to why these antinutritional components were lost during the soaking of the seeds. Regarding tannins, which are polyphenols that are found at the tegument level and whose reduction after seed soaking is ascribed to the impact of producing an ionic state (Fan *et al*., 2019; Bharadwaj *et al*., 2021). The modified ionic environment may alter the

integument's permeability, resulting in greater and quicker losses. Furthermore, phytasecatalyzed hydrolysis is responsible for the reduction in phytic acid throughout this soaking procedure (Chen *et al*., 2018). As a result of the hydrolysis of the phytate molecule, minerals are released for absorption by the intestine.

It is crucial to keep in mind however that when soaking, some nutritional compounds like minerals are lost. After a 24-hour soak, iron content of cowpea seeds was decreased from 6.60 mg/100 g DM to 5.68 mg/100 g MS, or 13.94 % loss (Martínez-Pineda *et al*., 2019). The minerals (calcium, potassium, iron, and magnesium) lost in the seeds are recovered in the soaking water (Liu *et al*., 2019). It is crucial to suggest food dipping as control measure of antinutritional factor while the operation time management prevents significant losses of water-soluble vitamins and minerals.

2.10.2 Autoclave and cooking

Autoclaving is typically utilized for heat treatments. The treatment stimulates the phytase enzyme and raises acidity in cereals and other plant-based diets (Ertop and Bektaş, 2018). When eaten following autoclaving, the majority of the foods demonstrated health benefits. For instance, cooking grains boosted their nutritious value by reducing the quantity of anti-nutrients (Arshad *et al*., 2023). Additionally, heating and soaking legume grains significantly reduced their phytic content (Shi *et al*., 2018). Beans foods are typically prepared in a pressure cooker or by boiling before being consumed. According to some research findings, boiling or heating foods significantly increased their nutritional value by corresponding removal of antinutritional components (such as trypsin and tannin inhibitors) (Patterson *et al*., 2017). According to a different study, boiling of legumes reduces their saponin and lectin concentrations and thus significantly improve their nutritional quality (Maphosa and Jideani, 2017). Additionally, the activity of trypsin

inhibitor in soybean was intensely reduced through roasting (Vagadia *et al*., 2017). It was also reported that soaking, heating, and autoclaving of legumes greatly decreased the amount of numerous antinutritional components (Torres *et al*., 2016). When compared to alternative processing techniques, the majority of prior studies found that autoclaving was the most effective way to decrease many antinutrient chemical contents (Abbas and Ahmad, 2018; Samtiya *et al*., 2020).

2.10.3 Milling

Milling is the oldest method for separation of the bran layer from the grains. It entails the transformation of grains into flour. The milling method removes anti-nutrients contained in grain bran, this includes lectin, tannin and phytic acid, but it has the significant limitation of also eliminating crucial minerals (Liu *et al*., 2017). For instance, milling research on millet showed that the milling process changed the chemical make-up of pearl millets. When pearl millet flour was baked, however, no significant changes were observed. However, the milling and heating processes used to make chapatti decreased the polyphenol and phytic acid contents while improving starch and protein digestion significantly digestion (Krishnan and Meera, 2018; Bassi *et al*., 2021). In a different study, two types of pearl millets were ground into bran rich section, semi refined and whole flour so as to assess the antinutrients, nutrients and mineral bioavailability. Except for the fat level, which was 1.3 %, the findings of the nutrient composition analysis did not reveal any differences between semi-refined flour and whole flour. As a result of the bran portion being removed, semi-refined flour was shown to have a lower phytate and oxalate level than whole flour (Kulthe *et al*., 2016).

2.10.4 Germination

Germination is defined as a procedure that includes actions that start with the matured dried seed absorbing water and end with a radicle, a piece of the embryo poking through the seed's envelopes (Ali and Elozeiri, 2017). Food's nutrient composition, biochemical makeup, and morphological makeup are regularly changed during germination. Thus, significant adjustments are made between the seeds and the culture medium during germination. Different varieties of grains and legumes reduce their phytate content during germination by 37 to 81 % (Patterson *et al*., 2017). Germination is another method that is considered to be particularly successful for reducing the antinutritional composition of plant-based foods (Nkhata *et al*., 2018). The effectiveness of this technique to lower phytate levels varies based on the material that was germinated, the germination circumstances, and most importantly the germination period. Additionally, the number of phytate-hydrolyzing enzymes and the germination conditions must be appropriate for the activity of the enzymes in order for germination to occur with the greatest efficiency.

Germination also results in significant decreases in phenolic component concentrations, which range from 9 to 56 % and especially in tannins, which decrease by 33 to 72 % (Diouf *et al*., 2019).

The presence of the specific enzyme phytase is what causes the reduction in phytates (Nissar *et al*., 2017). In fact, during seed germination, the various phytases found in the seeds act. During seed germination, the enzyme phytase is frequently activated, which reduces the content of phytic acid in the materials. Breakdown of phytates during germination can lessen their negative effects on mineral uptake. It can be started with a few easy procedures, such as washing the seeds to get rid of all the contaminants and soaking them in water for a few days. Usually, this method is used to reduce the

antinutritional content of cereals (Oghbaei and Prakash, 2016). In contrast to non-germinated cereals, which had decreased quantities of endogenous phytase enzyme activity, germinated cereals displayed increased activity of the phytase-degrading enzyme (Vashishth *et al*., 2017). There was a decrease in the phytic acid level to 23.95 % and 45.3 % respectively, following 72 and 96 hours of malting millet samples (Coulibaly *et al*., 2011; Samtiya *et al*., 2020). It was shown in a prior study by Bouajila *et al*. (2020) that measuring the phytate content of cereal-based grain samples 10 days after of germination resulted in a much lower level of phytate. Nkhata *et al*. (2018) revealed that samples of germinated buckwheat had higher levels of antinutrient components such (tannin, total phenolic and flavonoid contents). Recent research revealed that germination alters the isoflavone content of soybeans by activating -glucosidases; this improves the nutritional content since isoflavones are chelators (Yoshiara *et al*., 2018; de Camargo *et al*., 2019). The bioavailability of various minerals is raised in germinated cereals by reducing antinutritional factors such as phytic acid and tannin, that raises the nutritional value of the food items (Oghbaei and Prakash, 2016). According to Singh *et al*. (2017a), millets' germination yielded the highest decrease (75 %) in polyphenol concentrations in contrast to fermentation, soaking and microwave treatment.

2.10.5 Fermentation

An age-old technique for food preservation is fermentation. It is a normal process that starts when bacteria or yeast begin to break down the carbohydrates in diet. Despite the fact that accidentally fermented food is typically seen as rotten, controlled fermentation is frequently employed in the food manufacturing process. Yogurt, cheese, wine, beer, coffee, chocolate, and soy sauce are a few foods that are prepared through fermentation. Fermentation efficiently breaks down phytate and lectins in a variety of grains and legumes (Arbab Sakandar *et al*., 2021). Phytate and lectin content in grains and legumes

are significantly reduced during fermentation. This treatment method starts with enzymatic starch hydrolysis.

Generally, metabolites production, the breakdown of cyanogenic glucosides, enzyme generation, the production of a wide range of other compounds all depend on lactic acid bacteria and the development of probiotic qualities (Fleet and Zhao, 2018). The action of fermentative bacteria is thought to be responsible for the decrease in antinutritional levels during fermentation (Olagunju and Ifesan, 2021). Alpha-galactosides and phytates are reduced during fermentation, which increases the amount of iron and other minerals that are available. It encourages the ideal pH for the breakdown of phytic acid enzymes (Scheers *et al*., 2016; Mendoza-Avendaño *et al*., 2019). In grains and legumes, it lowers levels of some antinutritional ingredients, including phytates and α-galactosides; for millet, it lowers raffinose and phytic acid content by 83 % and 75 %, respectively (Diouf *et al*., 2019). Thus, the enzymatic and microbial process of fermentation significantly reduces antinutritional levels while simultaneously increasing the shelf life of food. The lowering of the pH makes this reduction role possible. The decrease rate attained by fermentation is higher in comparison to soaking; this suggests that utilizing fermentation to treat seeds and legumes, especially cowpea, would be beneficial to their nutritional value.

2.10.6 Extrusion cooking

Extruders were initially used for food preparation in 1869, which prompted extrusion cooking to become widely adopted (Bharadwaj *et al*., 2021). The extrusion cooking method creates a precooked product by fusing mechanical and thermal processes. This method raises the bioavailability of minerals to diminish antinutritional influences (Nikmaram *et al*., 2017). As a result, it enables a 30 % reduction in phytate levels (Wani and Kumar, 2016). Diouf *et al*. (2019) reported decrease in phytic acid of extruded seeds of green beans (*Phaseolus vulgaris*) and beans (*Vicia faba* L.) of 26.73 % and 20.75 %,

respectively. According to studies, certain phytic acid molecules are hydrolyzed into inositol tri, tetra and pentaphosphates during extrusion cooking process (Hirvonen *et al*., 2019).

The reduction of antinutritional factors is however, significantly influenced by the extrusion temperature and moisture of the substance to be extruded. During cooking of rice bran by extrusion at 140 ºC and 20 % humidity, phytic acid was reduced by 55.83 % (Bharadwaj *et al*., 2021). Less phytic acid breakdown in grain bran is caused by extrusion at low moisture contents. According to Bharadwaj *et al*. (2021), at 140 ºC, the average phytic acid concentrations in extruded rice brans were 19.92 mg/g at 14 % humidity, 18.63 mg/g at 14 % humidity, and 17.35 mg/g at 20 % humidity. As a result of these findings, it is feasible to recall that the extrusion temperature affects the antinutritional factor content more than the moisture content of the extruded product. Therefore, a humidity and temperature of 20 % and 140 °C respectively would be the ideal parameter pairs for lowering antinutritional elements in food products. Additionally, the method of extrusion cooking would be preferred over the other techniques covered above. In fact, unlike other processes like germination and soaking create a dissemination of these minerals into the water used for soaking, it makes minerals available without destroying them.

2.10.7 Steam pre-cooking

A standard method used in preparing some food items, including millet and rice, is steam pre-cooking. It is used to raise the nutritional value of rice (Swarnakar *et al*., 2022). Studies show that this process is used to reduce the quantities of antinutritional compounds in grain and legume seeds. In fact, rice grains, sunflower seeds, and beans all had their phytate levels decreased by 47.9 %, 55 %, and 52 %, respectively, by steam precooking. The "moin-moin" (seasoned steamed cowpea paste), making process

decreased tannins and phytate concentrations by 19.6-24.7 % and 7.8-14.0 %, respectively (Diouf *et al*., 2019). Phytic acid is thermolabile, which explains why phytate will reduce after heating because of its thermal degradation. According to Serna-Saldivar and EspinosaRamrez (2019), tannins are concentrated at grain tegument level but are eliminated during the thermal treatment procedure. The duration of the treatment and temperature are effective parameters for optimization in this thermal process. After 25 minutes of steam precooking at 100 °C, 110 °C and 115 °C, the average phytic acid concentration of untreated cereal bran was lowered from 38.14 to 18.72 mg/g,18.08 mg/g and 18.74 mg/g respectively (Diouf *et al*., 2019; Bharadwaj *et al*., 2021).

These results revealed that 110 °C for 25 minutes leads to the greatest (52.60 %) decrease in the phytic acid level. Thus, similar losses in antinutritional factors could be expected in the case of cowpea given that some of them are heat labile or are located in teguments where heat makes them more easily destroyed. Combining this treatment method with the other methods may result in greater reductions in antinutritional factors while preserving the final product's organoleptic and nutritional qualities.

2.11 Effect of Processing Methods on Cyanide Level

2.11.1 Fermentation

Fermentation is referred to as the chemical breakdown of substances, primarily by microbes. It is among the oldest methods of preserving food which has become popular in several nations because of its nutritive value and diversity of sensory qualities. By producing vitamins, vital amino acids, and the breakdown of antinutrients, fermentation increases the nutritional value of food (Nkhata *et al*., 2018). In Africa, lactic acid bacteria fermentation is a popular industrial technique. Faba beans were fermented by the lactic acid bacterium *Lactobacillus Plantarum* VTT E 133328 to determine if the chemical

change occurring during fermentation affect the antinutritional elements found in faba beans. Fermentation was found to be beneficial in lowering antinutritional elements present in faba beans (Sozer *et al*., 2019). Cassava roots that have been grated or soaked and which may or may not have been peeled are used in the fermentation process. This lowers the pH of the processed food. The cyanogen removal mechanisms used by the 2 types of fermentation result in differences in their efficacy. Western Africa and southern America damage countless plant cells by crushing, shredding, or grinding cassava parenchyma into little pieces, which helps linamarin and linamarase make good contact with one another. The remaining HCN gas is subsequently eliminated by roasting after the moist mash has been allowed to ferment for a number of days. This procedure considerably lowered the product's (garri or farinha) cyanogen content (Abiodun *et al*., 2020). Roots that have been grated conventionally are fermented using known bacteria (Phiri *et al*., 2020). Cyanogen glucosides can be effectively eliminated by fermenting grated cassava roots. 95 % of the linamarin were eliminated after 3 hours of grating (Ndubuisi and Chidiebere, 2018). Indrastuti *et al*. (2018) has shown that grating was primarily responsible for linamarin hydrolysis and that microorganisms contributed little to cyanogen reduction. Although grating quickly removes linamarin, products made from grated and fermented cassava roots still have substantial cyanide retention. In fact, 74 % and 40.3 % of the total cyanogens from the fermentation of grated cassava were still present after 3 and 8 hours, respectively. According to Indrastuti *et al*. (2018), the fermented paste still contained significant amounts of free cyanide and cyanohydrin, the stability of acidic pH of cyanohydrins may help to explain this (Cressey and Reeve, 2019). Thus, post-fermentation operations are critical for decreasing levels of free cyanide and cyanohydrin in finished products such as cassava couscous, garri (cassava flakes), placali (yucca), and agbelima (fermented cassava dough).

Roots that have been steeped in water and fermented are considerably more effective at reducing cyanogen than roots that have been granted. In fact, after three days of fermentation, more than 90 % of all cyanogens had been eliminated, and just about a third of the original linamarin remained. Neither cyanohydrin nor free cyanide accumulated in any substantial amounts (Ze *et al*., 2021). In this case, cyanogen removal depends on microbial development. Increased time of soaking and fermentation, as well as grating and peeling of cassava roots before soaking and fermentation steps, can increase the cyanogen elimination process (Chongtham *et al*., 2021). Atobrah (2020) reported that soaking cassava roots for six days, shredding them on day six, and fermenting the resulting mash for four days to make farina allowed for a 98 % elimination of cyanide.

Long-term soaking may induce mold spores, fungi and unwanted microorganisms into finished items (Jaber and Enkerli, 2016; Ježek *et al*., 2020). The mold, which usually has no adverse effects, helps weaning foods have less stickiness. It is believed that cooking renders the harmful germs harmless.

Cyanogens can also be eliminated through dry fermentation. According to Montagnac *et al*. (2009), after cassava roots underwent dry fermentation (solid state fermentation), 89.6 % of the cyanogens in ugali (maize flour porridge) were lost. A range of 12.5 to 16.5 % of the cyanide was also found to be retained in cassava roots that had undergone heap fermentation (Onyango, 2019; Lebot, 2019).

2.11.2 Boiling

The removal of cyanogen from diverse plant food products by boiling or cooking has been shown to be effective, but the outcomes vary depending on the length of processing and the species of plant. According to a number of studies, boiling and cooking are two of the best methods used to decrease cyanogens in food plants. These actions seem to encourage cell wall rupturing, allowing the transportation of cellular contents such as toxins and

antinutrients (Olawoye, 2016; Nyirenda, 2020). According to a study on the bamboo plant, it was found that after boiling the *Bambusa vulgaris* shoots for 10 minutes, the level of cyanogenic glycoside was reduced by 67.84–76.92 %. Up to 87 % less cyanogen was removed from the shoots after boiling them for an additional 10 minutes (Karanja, 2017). Similar experiments with cassava found that boiling small pieces of cassava in a lot of water significantly increases the effectiveness of boiling approach for the reduction of cyanogen (Ismaila *et al*., 2018; Seth, 2020). However, according to other research, boiling can only lower the cyanogen level by 50 %, making it ineffective as a cyanide removal procedure. The high temperatures used in this processing technology are to blame for its ineffectiveness. Linamarase, a heat-labile -glucosidase, is denatured at 100 ^{o}C, which prevents linamarin from hydrolyzing into cyanohydrin. Nyirenda, (2020) reported that after 25 minutes of boiling, bound glucosides were decreased to 45 and 50 %. It is reported that a heat labile glycosidase called linamarase, is denatured at a high temperature of 100 °C, preventing linamarin from being degraded into cyanohydrin and subsequently HCN. According to a study by Nyirenda (2020), after 25 minutes of boiling, bound glucoside was decreased to 45 – 50 %. After boiling, cyanohydrin and free cyanide in the roots of cassava are reduced to trace quantity. Ndubuisi and Chidiebere (2018) reported that boiling of 50 g cassava roots resulted in 6 % free cyanide and cyanohydrin content from the total cyanogens content and only 3 % in small pieces (2 g). Additionally, according to Panghal *et al*. (2019), free cyanide and cyanohydrin were observed to be volatilized after boiling, thereby decreasing its content in cooked cassava roots. For instance, Kolapo *et al*. (2021) observed that by lowering the size of the cassava chips, boiling 50 g and 2 g pieces of cassava root for 30 min each led to a 25 and 75 % reduction respectively in the amount of cyanide present respectively. Similar to this, cyanogen retention was decreased from 70 % to 24 % by multiplying the amount of water by a factor of 1 to 5. In contrast to enzymatic degradation, Jeelani *et al*. (2018) revealed that solubilizing cyanogenic

glucosides from small cassava chips into the large amount of water may account for the more effectively removal of cyanogen.

2.11.3 Roasting

Roasting is a common method of food preparation in the home. To achieve a wellbrowned exterior and a moister cooked interior, roast in an uncovered pan without water. For cereals, fruits, and vegetables, dry heat (roasting) is a popular processing technique that is known to increase nutrient availability, inhibit inactive enzymes that hasten nutrient destruction, and eradicate unfavorable bacteria and food toxins (Hwang *et al*.,

2007). Furthermore, the physical properties of food, such as color, texture, and flavor, are altered, and roasting method also has conflicting effects on the food's nutritional content

(Biglar *et al*., 2012). This method works best with sweet cassava, which is popular in the South Pacific and has little cyanide (Fukushima *et al*., 2016). According to Nwafor *et al*. (2017), The nutritional value of food plants has increased and seeds of *Adenanthera pavonina* L. (Fabaceae) have become more successful in removing anti-nutritional elements. *Adenanthera pavonina* L. (Fabaceae) seeds had a drop in HCN of 83.2 %, from 377 mg kg^{-1} - 63.5 mg kg^{-1}. Additionally, Moknatjou *et al*. (2015) reported that roasting flaxseed significantly reduced its HCN content. This reduction could be brought on by the hydrolysis-induced production of HCN, the evaporation of HCN following hydrolysis, or both. Evaporation of HCN was corroborated by the 5.7 % loss of moisture in flaxseed after four minutes of roasting, which was larger than the 3.9 and 1.4 % achieved after autoclaving and six pelleting, respectively. The strong heat induction power of roasting may contribute to this water loss. Heat facilitates the production of HCN alongside the rearranging of charge groups brought on by the interaction between roasting and material. Then, it becomes feasible to vaporize this flammable HCN, leading to HCN reaction.

Due to the steady removal of cyanohydrin and free HCN into the environment, which results in a small amount of free HCN (3.4 mg/kg) and cyanohydrin (2.2 mg/kg), the roasting process used to make garri from grated cassava after fermentation is relatively effective (Indrastuti *et al*., 2018). Roasting reduces the total cyanide concentration of foods from plants significantly, but not as effectively as boiling. As a result, wet heating (boiling) is more effective than dry heating (roasting) in cyanide removal (Ubwa *et al*., 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Sources of plants seeds

Cassava seeds with identification numbers IBA070539 and IBA011368 were obtained from International Institute of Tropical Agriculture (IITA), located in Ibadan, Oyo State.

3.1.2 Reagents and chemicals

Petroleum ether, sulphuric acid, boric acid, ammonia, hydrochloric acid, $KMNO_4$, Na_2SO_4, $CuSO_4$, selenium, NaOH, NH_3, NH_4OH. All the reagents and chemicals were of GPR grade and were produced by Sigma Aldrich Chemical Company Incorporation in Milwaukee, Wisconsin, USA, and British Drug House (BDH) Limited in England.

3.2 Methods

3.2.1 Preparation of cassava seed flour

Cassava seedlings were weighed, cleaned, and sorted to remove stones and foreign materials. The seeds were oven dried at 45 °C till they reached a constant weight, then cooled in a desiccator. Dried seeds were ground to fine powder using an analytical mill (Cole Parmar, IL, USA) at a high speed of 20,000 rpm and stored in an airtight container for further analysis.

3.2.2 Processing methods

3.2.2.1 Fermentation

A total of 59 grams of milled cassava seeds were weighed into three different plastic containers. The samples were then drained, covered in nylon and packed in jute sacks after being soaked in water for two minutes. They were fermented for 5 days in a dark and warm place (30 – 32 °C). samples were taken daily from the third to the fifth day, air-dried, and stored in airtight plastic bags until they were needed for further analysis. ***3.2.2.2 Boiling***

Boiling was done according to the method described by Makinde and Akinoso (2014). Exactly 59 grams of the milled cassava seeds were boiled inside a pot with tap water at 1:3 (w/v), for different times of 1 hour, 2 hours, 18 hours, 20 hours, 22 hours, 24 hours

and 26 hours. The boiled samples were dried, then stored in airtight plastic bags until they were required for further analysis.

3.2.2.3 Roasting

Roasting was carried out using the method of Mohamed *et al*. (2007). Three sets of milled cassava seeds (100 grams each) were roasted in an oven at 180 ^{o}C for 30, 40, and 50 minutes, respectively, and then stored in airtight plastic bags until needed for further analysis.

3.2.3 Defatting of the cassava seed flour

Milled cassava seed flour weighing a total of 100 grams was put into a thimble, transferred to the Soxhlet chamber, and then 500 ml of solvent (petroleum ether) was put in the flat-bottom flask of Soxhlet extractor for distillation. Following extraction, the produced defatted flour was air-dried to remove any remaining solvent, then sieved to remove any larger grains or particles, forming a powder. The defatted sample was stored in air-tight plastic bag for further analysis.

3.2.4 Analysis of cassava seed flour samples

3.2.4.1 Proximate analysis

The moisture, crude fibre, ash, oil and carbohydrate contents were all carried out using the methods as described by AOAC (2012). All analysis were done in triplicate

3.2.4.2 Determination of protein content

To determine the content of crude protein in cassava seed sample, the Kjeldahl method was used. 2 g of sample was weighed and wrapped in Whatman filter paper (N0.1) before being placed in a Kjeldahl digestion flask with the addition of sulphuric acid (10 ml). A

catalyst mixture (10:5:1) of sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$), and selenium oxide (SeO_2) were added to the flask in the amount of 0.5 g to aid digestion. Four pieces of anti-bumping granules were added. After three hours in a Kjeldahl digestion apparatus, the contents of the flask turned pale green. The digested sample was cooled and diluted with distilled water to a volume of 100 ml in a standard volumetric flask. An aliquot (10 ml) of the diluted solution was then transferred to a Markham distillation apparatus with 10ml of 45 % sodium hydroxide and distilled into 10 ml of 2 % boric acid containing 4 drops of methyl red indicator until 70 ml of distillate was obtained. The distillate was then titrated with 0.01 N hydrochloric acid until it turned gray.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times v \times 100}{W \times C} \quad \ldots\ldots (2)$$

Where:

a = Titre value of the digested sample

b = Titre value of blank sample v =

Volume after dilution (100ml) W =

Weight of dried sample (mg)

C = Aliquot of the sample used (10ml)

14 = Nitrogen constant in mg

% Protein = % Nitrogen x 6.25 (Conversion factor)

3.2.4.3 Determination of oil content

The oil content of cassava seed sample was determined by ether extract method using Soxhlet extractor apparatus. A total of 2 g of the sample was placed in a known weight fat free thimble (W_1) after being wrapped in a filter paper. The thimble and sample's weight were determined (W_2). The sample-filled thimble was put into a soxhlet extractor. 500 ml round bottom ground joint flask containing 300 ml petroleum ether was set on a heating mantle. After 24 hours of heating and extraction, the thimble containing the content was removed, dried in an oven at 500 ^oC for 24 hours, cooled in a desiccator, and weighed (W_3).

The % lipid content of the sample was calculated as follows:

$$\% \text{ Oil} = \frac{100 - (W_2 - W_3)}{W_2 - W_1} \quad \ldots\ldots (3)$$

3.2.4.4 Carbohydrate content determination

The difference described according to AOAC (2012) was used to determine the total carbohydrate content. Each sample's total content of crude protein, fat, moisture was added and subtracted from 100. The result obtained was the percentage carbohydrate content of the samples.

% Carbohydrate = 100 – (% Moisture + % Ash + % Protein + % Oil) ….. (4)

3.2.4.5 Determination of moisture content

The moisture content was used to determined using the oven drying method. A total of 2 g of sample was accurately weighed in a clean dry crucible (W_1). The crucible was then

placed in an oven at 100 to 105 ^{o}C for 6 to 12 hours to obtain a constant weight. The crucible was reweighed (W_2), and the percentage moisture was calculated using the formula.

$$\% \text{ Moisture} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100 \quad \ldots\ldots (5)$$

3.2.4.6 Crude fibre

The fibre content was determined using the method described by AOAC (2012).

% Crude Fibre = Loss in weight after incineration x 100

3.2.4.7 Ash content determination

The ash content in cassava seed flour was determined using the method of AOAC (2012). A balance was used to weigh the empty crucible and the 5 g of sample inside the crucible. The sample was then incinerated in a furnace at 600 °C for eight hours (Ashing Muffle Furnace, CARBOUTE, UK). After incineration, the sample was allowed to cool in the desiccators. The final weight of the crucible with ash was determined. The percentage of ash was calculated using the following formula:

$$\% \text{ Ash} = \frac{\text{Weight of Ash}}{\text{Initial weight of the sample in dry matter}} \times 100 \quad \ldots\ldots (6)$$

3.3 Amino acid profile

The amino acid profile of the sample was determined using the method described by AOAC (2005) with modifications. The sample was loaded into the PTH Amino Acid Analyzer by Applied Biosystems after being dried to a constant weight of 70 ^{o}C, defatted, hydrolyzed, and evaporated in a rotary evaporator.

3.3.1 Computation of amino acid groupings

The computation of the total amino acid (TAA), total essential amino acids (TEAA), total nonessential amino acids (TNEAA), Total Sulphur amino acids (TSAA), Total nonSulphur amino acids (TNSAA), Total aromatic amino acids (TArAA), Total nonaromatic amino acids (TNArAA), were calculated as described by Ibegbulem *et al.* (2013).

3.3.2 Determination of essential amino acid scores

The essential amino acid scores of cassava seed protein concentrates were calculated using the FAO/WHO (2007) provisional scoring pattern for preschool children (1-2years) as the ratio of the actual amount (mg) of each essential amino acid per gram of protein to the required amount (mg) of that essential amino acid per gram of a reference protein, as described by Nielsen (2001).

3.4 Determination of functional properties cassava seed protein concentrate

3.4.1 Determination of bulk density

The method described by Onwuka (2005) was used.

$$\text{Bulk Density (g/ml)} = \frac{\text{Weight of sample (g)}}{\text{Volume of sample (ml)}} \quad \ldots\ldots (7)$$

3.4.2 Swelling capacity and solubility index

The swelling capacity and solubility index was determined as described by Onwuka (2005). The residue was represented by the amount of water solubilized.

$$\text{Solubility} = \text{Weight of after drying supernatant} \times 100 \quad \ldots\ldots (8)$$

Weight after drying of dried sample

3.4.3 Water and oil absorption capacity

The water and oil absorption capacity was determined as described by Onwuka (2005). A total of 2 g of the sample was mixed with 10 ml of distilled water or oil for 5 minutes on a magnetic stirrer. The mixture was centrifuged at 3500 rpm for 30 minutes, and the volume of the supernatant was measured using a 10 ml measuring cylinder on each sample. The water density was taken to be 1 g/ml.

$$WAC = \frac{\text{Volume of water absorbed X 100}}{\text{Weight of sample used}} \quad \ldots\ldots (9)$$

3.4.4 Determination of gelation capacity

The method described by Onwuka (2005) was used for the determination of gelation capacity. Test tubes were filled with a sample suspension that ranged from 2 % to 20 % (w/v) in distilled water. The samples in the test tubes were then heated for one hour in a boiling water bath before being rapidly cooled under cold running tap water. The test tubes were then cooled for another 2 hours at 4 °C. The concentration with the least gelation was determined when the sample from the inverted test tube did not fall or slip.

3.4.5 Viscosity measurement

The viscosity was determined using the rotating spindle method that is given in the Encyclopaedia of Industrial Chemical Analysis (E.I.C.A). Using a Brookfield dial viscometer, the sample's viscosity was determined. In a single-use plastic cup, 100 ml of water was added to a 5-gram sample of cassava seed flour. A water bath was used to heat the cup's contents to boiling point. After that, the cup was cooled to room temperature, or roughly 25 °C. The disposable cup with the sample inside was set underneath the apparatus. Dial reading was obtained and recorded using spindle number 2 with a speed of 30 rpm. The actual viscosity reading in millipascal-second (mpa.s) was calculated with the formula:

Actual viscosity reading (AVR) = M multiply by 10

Where M= Dial reading

3.4.6 Foaming capacity

The method described by Onwuka (2005) was used for the determination of foaming capacity. A total of (3.0 g) of the sample was weighed and 50 ml of distilled water was added in a graduated cylinder at 30 ± 2 ºC. The suspension was mixed and shaken to foam for five minutes. The foaming capacity was calculated as the volume of foam after 30 seconds of whipping. To calculate the percentage of the initial foam volume that remained after an hour of whipping, the volume of foam was measured.

$$FC\ (\%) = \frac{(AW - BW)\ X100}{BW} \quad \ldots\ldots\ (10)$$

Where, AW = after whipping, BW = before whipping

3.5 Preparation of Cassava Seed Protein Concentrate

The method of Chandi and Sogi (2007) was employed to prepare cassava seed protein concentrate with slight modification to the drying method, centrifugation speed and time. The defatted cassava seed flour was dried overnight in a fume hood to remove residual solvent, sieved through 100-ml sieve and stored in a plastic bag at 5 °C. The defatted flour was suspended in distilled water separately (1:10). After being raised to pH 9 (using 4 M NaOH solution), the slurry was adjusted to pH 4.5 (using 4 M HCl) and centrifuged at 3000 g for 20 minutes. The precipitate was extracted twice by constant stirring with distilled water (1:2 w/v) adjusted to pH 7 with 4 M NaOH, followed by centrifugation at 3000 g for 20 minutes. The protein concentrate was stored in vacuum-sealed bags at 5 °C from where the sample was drawn for analysis.

3.6 Determination of Percentage Yield and Recovery of Cassava Seed Protein Concentrate.

The percentage yield of cassava seed protein concentrate was calculated using the formula:

$$\%\ Yield = \frac{\text{Weight of protein concentrate (g)} \times \%\ \text{protein in protein concentrate} \times 100}{\text{Weight of seed flour (g)}} \quad \ldots\ldots\ (11)$$

$$\%\ Recovery = \frac{\text{Weight of protein concentrate (g)} \times \%\ \text{protein in protein concentrate} \times 100}{}$$

Weight of seed flour (g) x Protein in seed flour ….. (12) **3.7**

Data Analysis

Statistical analysis was performed using SPSS version 2.2.0. T-test and one way analysis of variance (ANOVA) tools will be used where required. All data will be analyzed at a 95 % confidence interval and values will be considered statistically significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Proximate composition

The proximate compositions of whole and defatted cassava seed flour (DCSF) are shown in Table 4.1. The moisture content of whole cassava seed flour (9.89 ± 0.16 %) was significantly higher ($p<0.05$) than that in the defatted cassava seed flour (5.54 ± 1.45 %).

This trend was different for the ash content which was significantly higher ($p<0.05$) in the DCSF than in the whole cassava seed flour. The whole cassava seed was rich in oil (20.75 %) while only residual amount was found in the DCSF. The crude protein in the DCSF was significantly higher ($p<0.05$) than in the whole cassava flour. However, the crude fibre content in the whole and the defatted cassava seed flours were not significantly different ($p>0.05$) but carbohydrate contents were significantly different ($p<0.05$).

Table 4.1: Proximate Compositions of Flours from Whole and Defatted Cassava Seeds

Proximate constituents	Whole cassava seed flour composition (%)	Defatted cassava seed flour composition (%)
Moisture	9.89 ± 0.16^{b}	5.54 ± 1.45^{a}
Ash	4.20 ± 0.23^{a}	7.03 ± 1.08^{b}
Oil	20.75 ± 2.66^{b}	2.19 ± 0.32^{a}

Crude protein	16.64 ± 0.46^{a}	20.75 ± 0.37^{b}
Crude fibre	11.94 ± 2.10^{a}	13.94 ± 1.87^{a}
Carbohydrate	35.03 ± 5.73^{a}	49.99 ± 1.20^{b}

Values are means of triplicate determinations ± SD. Values along rows with different superscripts are significantly ($p \leq 0.05$) different

4.1.2 Yield and recovery of protein concentrate

The percentage protein yield and recovery for cassava seed were 26.67 % and 84.31 %.

4.1.3 Functional properties of cassava seed protein concentrate

The result of functional properties of cassava seed protein concentrate is shown in Table 4.2. The Bulk density obtained in this study (0.75 ± 0.78 g/ml) was lower than the bulk density obtained for protein concentrates of some seeds. Swelling capacity (277.18 ± 16.97 %) was higher than the water absorption capacity obtained (250.77 ± 16.90 %) and oil absorption capacity (209.81 ± 4.64 %). In addition, solubility index (6.68 ± 1.04 %)

and foaming capacity (5.50 ± 2.12 %) were lower than least gel capacity (17.00 ± 1.15 %) and viscosity obtained (56.25 ± 4. 65 mpa.s).

Table 4.2: Functional Properties of Cassava Seed Protein Concentrate

Parameter	Functional properties
Bulk density (g/ml)	0.75 ± 0.78
Water absorption capacity (%)	250.77 ± 16.90
Oil absorption capacity (%)	209.81 ± 4.64
Swelling capacity (%)	277.18 ± 16.97
Solubility index (%)	6.68 ± 1.04

Least gel capacity (%)	17.00 ± 1.15
Foaming capacity (%)	5.50 ± 2.12
Viscosity (mpa.s)	56.25 ± 4.65

Values are means of triplicate determinations ± SD

4.1.4 Amino acid composition of cassava seed protein concentrate

A total of 18 amino acids were identified in cassava seeds protein concentrate (Table 4.3), consisting of 9 essential amino acid (Figure 4.1) and 9 non-essential amino acid (Figure 4.2). The total amino acid content was 79.88 ± 7.99 g/100g (Table 4.5), the cassava seed was rich in amino acid. Generally, glutamic acid (13.78 ± 2.15 g/100g) was the highest amino acid while tryptophan (0.83 ± 0.99 g/100g) was found to be the least amino acid (Table 4.3). The concentration of the total non-essential amino acid was 48.75 ± 3.49 g/100g, glutamic acid remained the highest while cystine (1.58 ± 0.68 g/100g) was the least (Figure 4.1). For essential amino acids, the total essential amino acids with histidine was 29.76 ± 2.56 g/100g and total essential amino acids without histidine was

27.55 ± 2.51 g/100g. Leucine was the highest amino acid (6.57 ± 0.62 g/100g) while tryptophan was the least amino acid (0.83 ± 0.99 g/100g) (Figure 4.2). The concentration of total Sulphur-containing amino acid was 2.90 ± 0.85 g/100g while concentration of total aromatic amino acid was 7.22 ± 0.02 g/100g (Table 4.4). Sulphur containing amino acids (methionine and cystine) were similar in concentration (1.32 ± 0.17 g/100g and 1.58 ± 0.68 g/100g respectively) (Figure 4.3), for aromatic amino acids, phenylalanine (3.46 ± 0.13 g/100g) was the highest while tryptophan was the lowest (0.83 ± 0.99 g/100g). Finally, the amino acid score showed that lysine was the limiting amino acid (amino acid score = 0.88) (Table 4.5).

Table 4.3: Amino acid Composition of Cassava Seed Protein Concentrate

Amino Acid	**Concentration (g/100g protein)**
Alanine	5.38±0.08
Arginine	5.26±0.26
Aspartic Acid	9.40±0.44
Cystine	1.58±0.48
Glycine	3.67±0.48
Glutamic Acid	13.78±1.52
Histidine	2.21±0.04
Isoleucine	3.79±0.22
Leucine	6.57±0.44

Lysine	4.60±0.97
Methionine	1.32±0.12
Phenylalanine	3.46±0.09
Proline	4.23±0.77
Serine	3.92±0.30
Threonine	2.92±0.09
Tryptophan	0.83±0.07
Tyrosine	2.93±0.18
Valine	4.08±0.22

Values are means of triplicate determinations ± SD

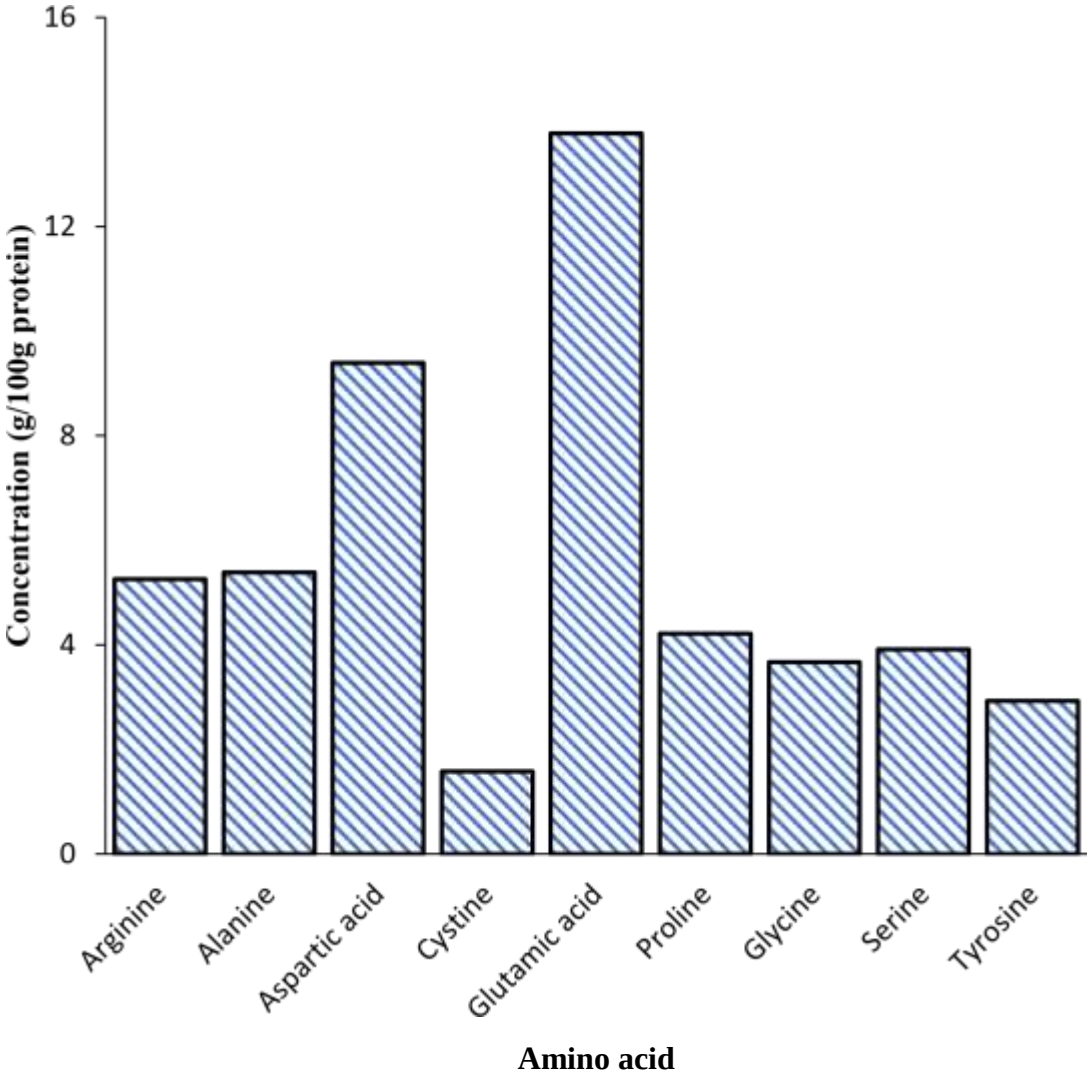


Figure 4.1: Non-essential Amino acid Composition of Cassava Seed Protein Concentrate

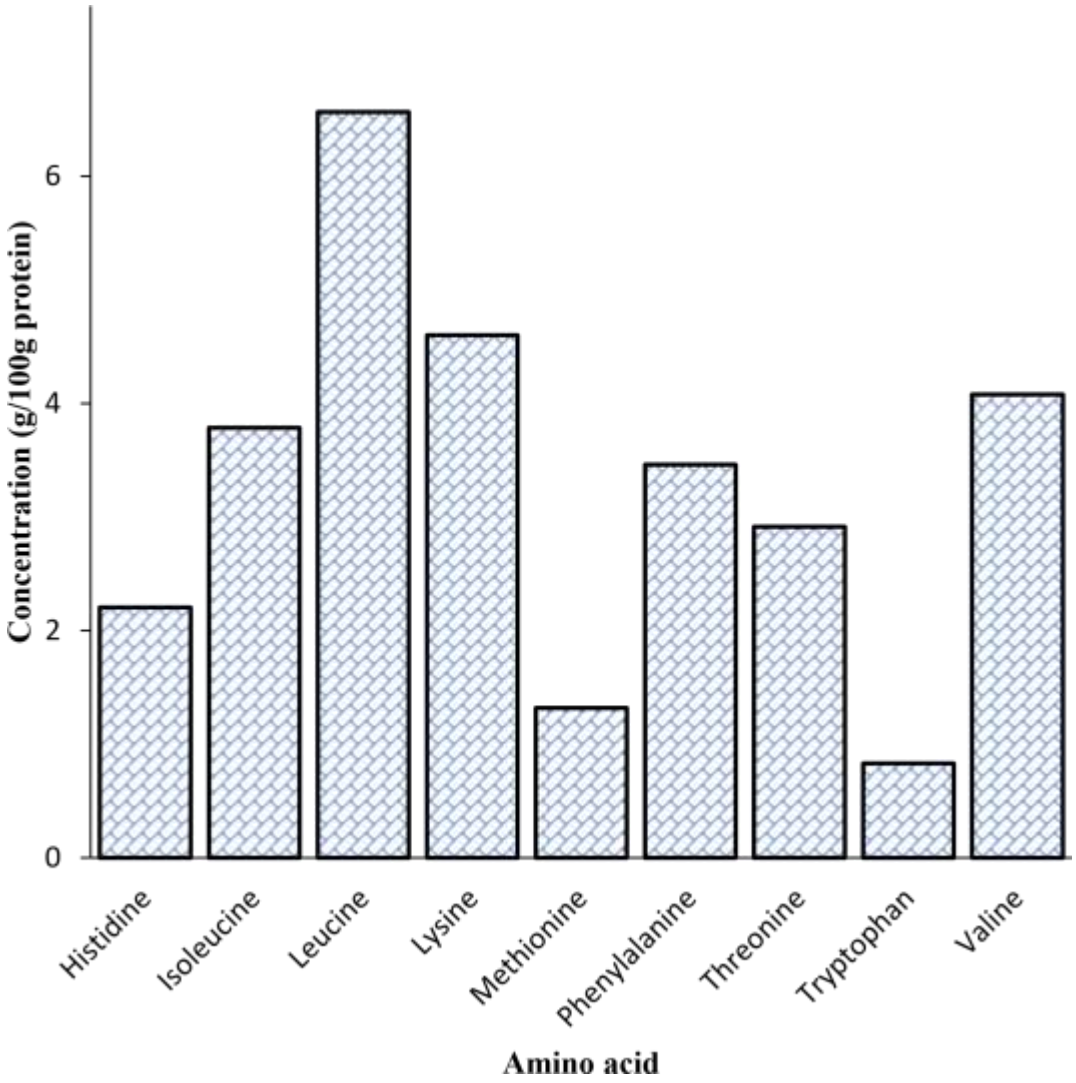


Figure 4.2: Essential Amino acid Composition of Cassava Seed Protein Concentrate

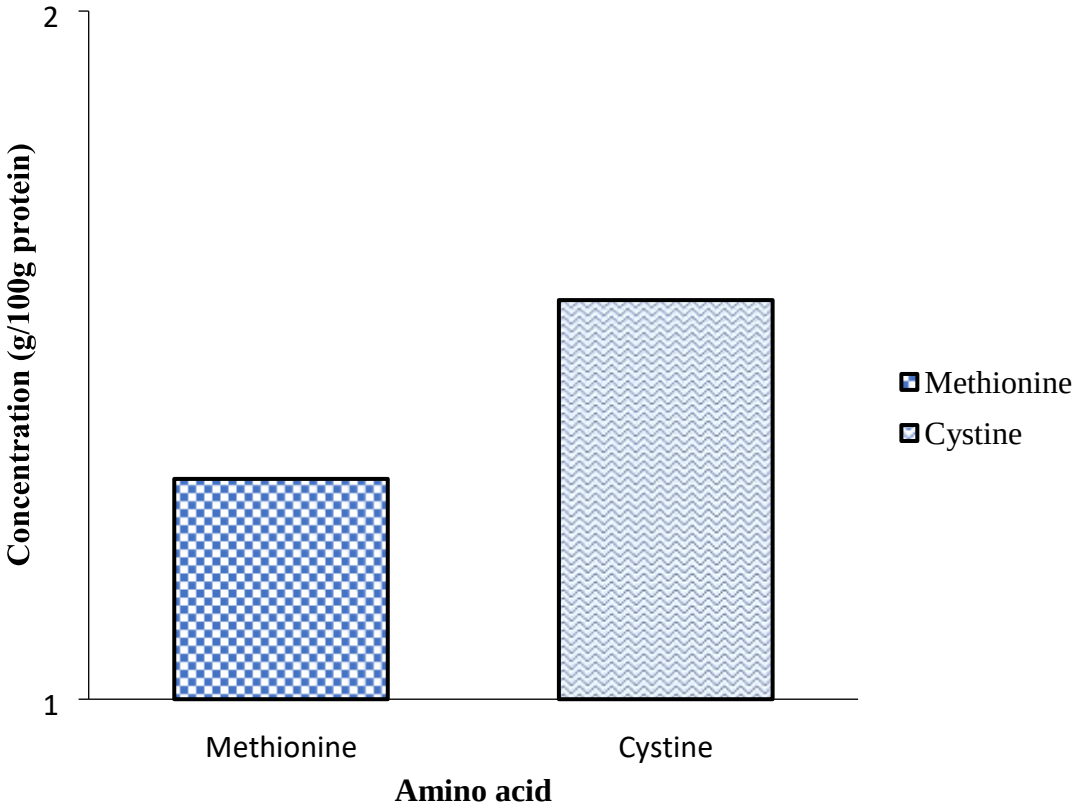


Figure 4.3: Sulphur Containing Amino Acid Composition of Cassava Seed Protein Concentrate

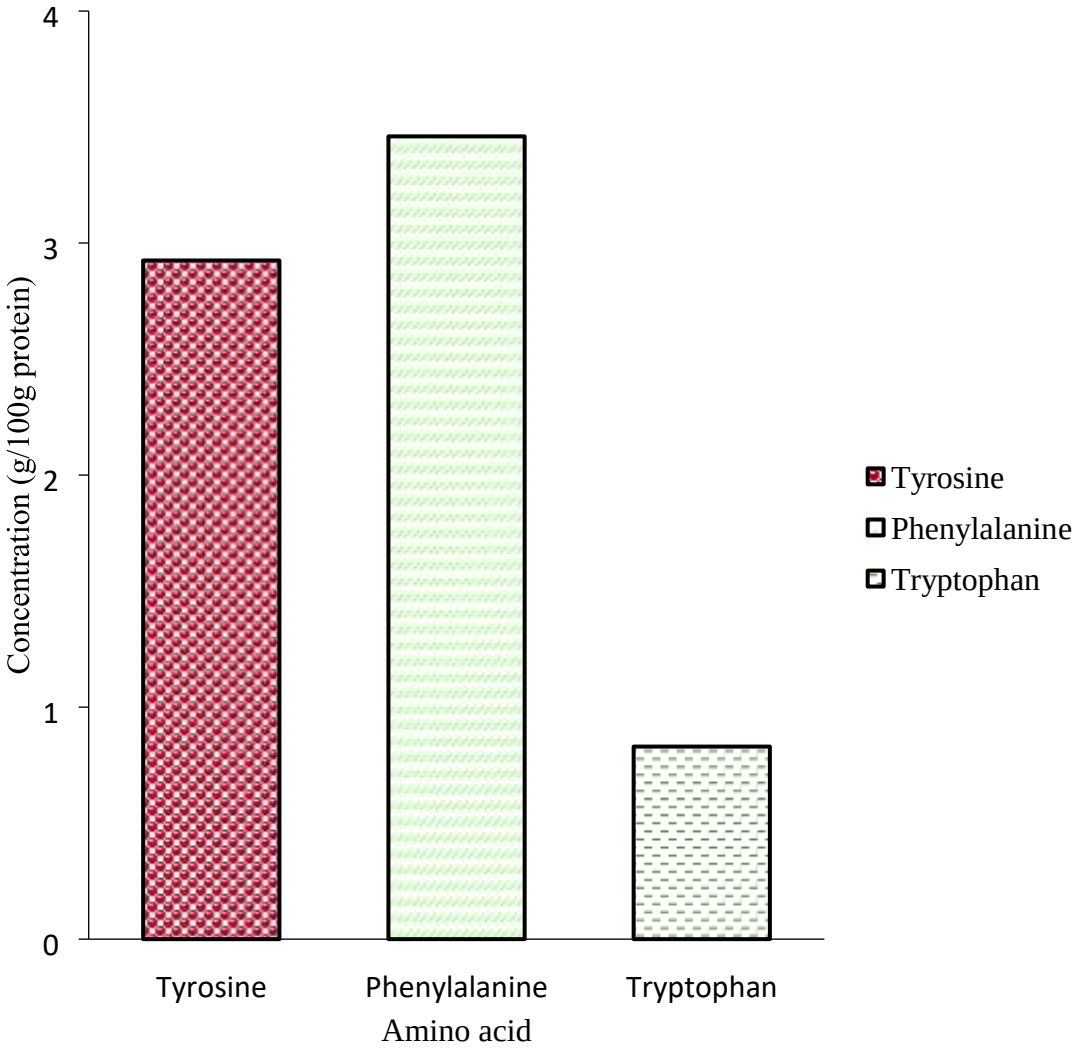


Figure 4.4: Aromatic Amino acid Composition of Cassava Seed Protein Concentrate

Table 4.4: Classification of Amino acids in Cassava Seed Protein Concentrate

Amino acid	Concentration (g/100g protein)
Total AA	79.88
TNEAA	48.75
TEAA with HIS	29.76
TEAA without HIS	27.55
TSAA	2.90
TNSAA	76.98
TarAA	7.22
TNArAA	72.67

AA-Amino acid, TNEAA-Total Non-essential amino acid, TEAA-Total essential amino acid, TSAA-Total Sulphur amino acid, TNSAA- Total non-Sulphur amino acid, TArAATotal aromatic amino acid, TNArAA-Total non-aromatic amino acid, HIS-Histidine **Table 4.5: Amino acid Score of Essential Amino acids in Cassava Seed Protein**

Concentrate

Essential Amino Acid	Concentration (g/100g protein)	FAO/WHO (2007) Pre-school(1-2years) in g/100g protein	**Amino acid score
Histidine	2.21	1.80	1.23

Isoleucine	3.79	3.10	1.22
Leucine	6.57	6.30	1.04
Lysine	4.60	5.20	***0.88**
Methionine + Cysteine	2.90	2.50	1.16
Phenylalanine + Tyrosine	6.39	4.60	1.39
Threonine	2.92	2.70	1.08
Tryptophan	0.83	0.70	1.19
Valine	4.08	4.10	1.00
Total	34.29	31.0	

* Lysine is limiting amino acid (Amino acid score = 0.88)

**Calculated based on FAO/WHO (2007). Suggested Pattern of amino acid requirements for preschool children (Age 1-2 years)

4.1.5 Anti-nutrient composition of whole and defatted cassava seed flour cassava seed protein concentrate

Anti-nutrient compositions in whole cassava seed, defatted cassava seed flour (DCSF) and cassava seed protein concentrate are shown in Table 4.6. The level of flavonoid

(10.50 ±1.17 mg/100g), alkaloid (8.38 ± 1.11 mg/100g), oxalate (2.38 ± 0.10 mg/100g), phytate (3.66 ± 0.25 mg/100g) and cyanide (1191.24 ± 158.71 mg/100g) were significantly higher ($p<0.05$) in the whole cassava seed flour, than both DCSF and protein concentrate. These values are similar in both DCSF and protein concentrate. Saponin and phytate concentrations were not significantly different ($p>0.05$) in the whole (1.77 ± 0.16 mg/100g and 3.66 ± 0.25 mg/100g respectively) and defatted sample (1.54 ± 0.21 mg/100g and 3.50 ± 0.31 mg/100g respectively), but were significantly higher ($p<0.05$) in the protein concentrate. There was no significant difference ($p>0.05$) in tannins in the three samples.

Table 4.6: Anti-nutrient Composition of Whole and Defatted Cassava Seed Flours and Protein Concentrate

Anti-nutrient (mg/100g)	Whole seed flour	Defatted seed flour	Protein concentrate

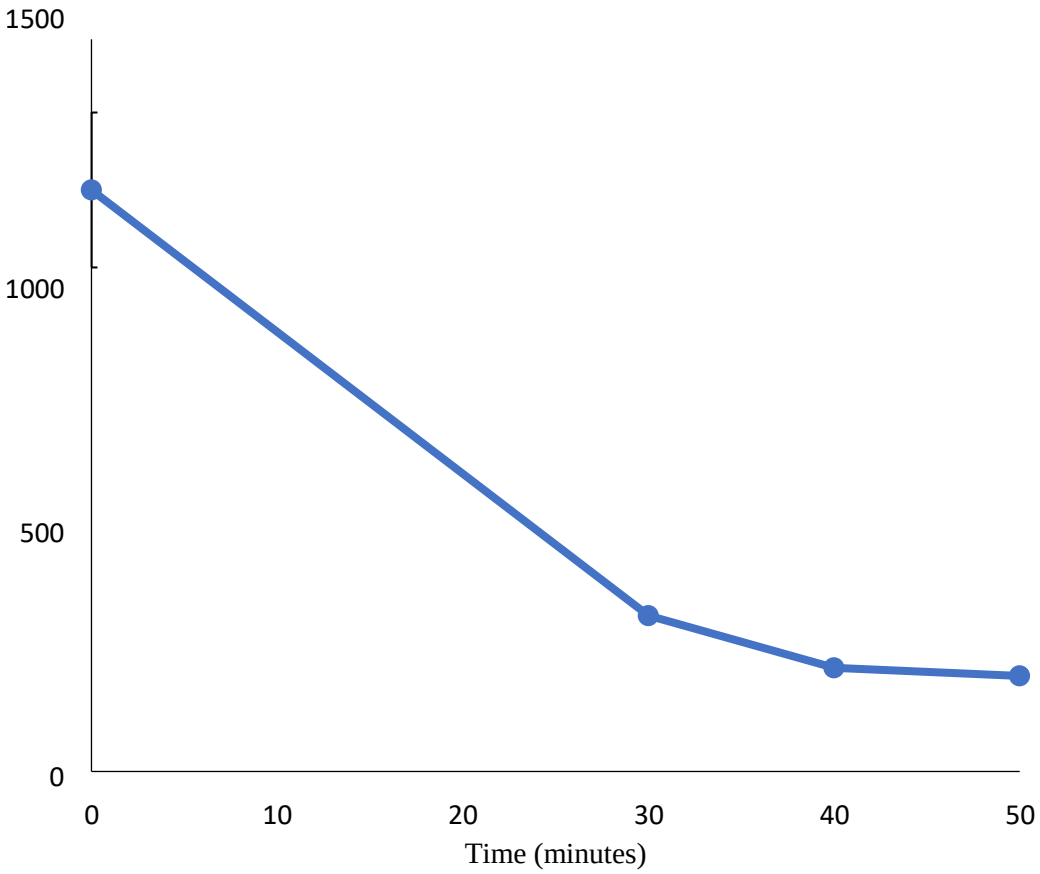
Flavonoids	10.50 ±1.17^{b}	2.00 ± 0.02^{a}	2.60 ± 0.70^{a}
Alkaloids	8.38 ± 1.11^{b}	3.75 ± 0.29^{a}	3.02 ± 0.02^{a}
Saponin	1.77 ± 0.16^{b}	1.54 ± 0.21^{b}	0.83 ± 0.21^{a}
Phytate	3.66 ± 0.25^{b}	3.50 ± 0.31^{b}	1.56 ± 0.44^{a}
Oxalate	2.38 ± 0.10^{c}	1.85 ± 0.26^{b}	0.87 ± 0.08^{a}
Tannin	342.10 ± 36.99^{a}	312.30 ± 7.87^{a}	320.30 ± 6.47^{a}
Cyanide	1191.24 ± 158.71^{b}	1141.35 ± 16.16^{a}	1008.43 ± 9.30^{a}

Values are means of triplicate determinations ± SD. Values along rows with different superscripts are significantly ($p \leq 0.05$) different

4.1.6 Effects of processing on cyanide composition of cassava seed protein concentrate

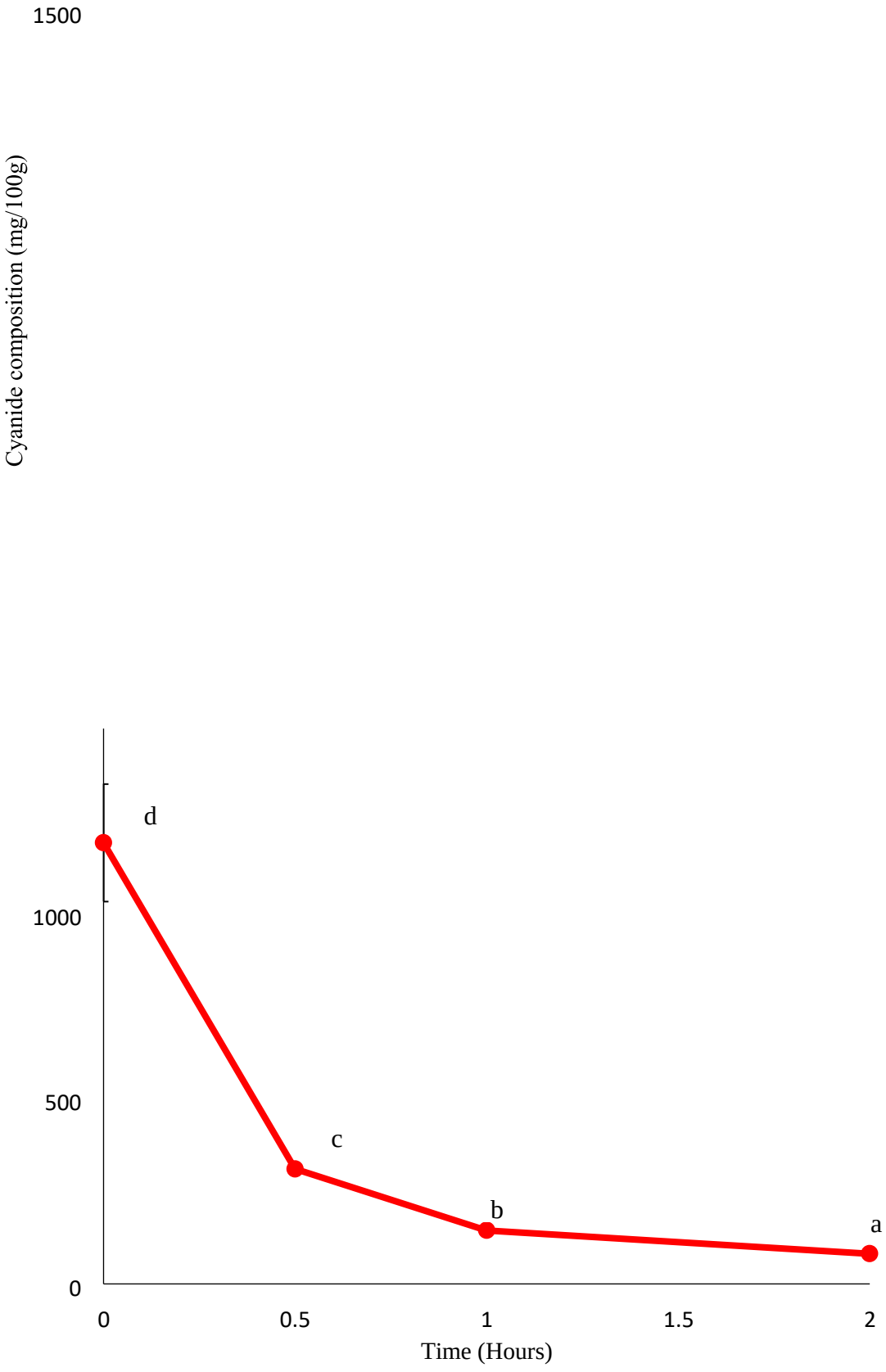
The effects of three processing methods (roasting, boiling and fermentation) on the cyanide level of cassava seeds are shown in figures 4.5-4.7. Cyanide content in protein

concentrate was reduced after 50 minutes of roasting from 1008.43 ± 9.30 mg/100g to 195.70 ± 1.17 mg/100g (Figure 4.5), 2 hours of boiling (81.35 ± 2.31 mg/100g) (Figure 4.6) and five days of fermentation (213.01 ± 0.11 mg/100g) (Figure 4.7), compared to cyanide level in the whole cassava seeds (1191.24 ± 158.7 mg/100g).



Values are means of triplicate determinations ± SD

Figure 4.5: Effect of Roasting Time on Cyanide Composition of Cassava Seed Protein Concentrate



Values are means of triplicate determinations ± SD

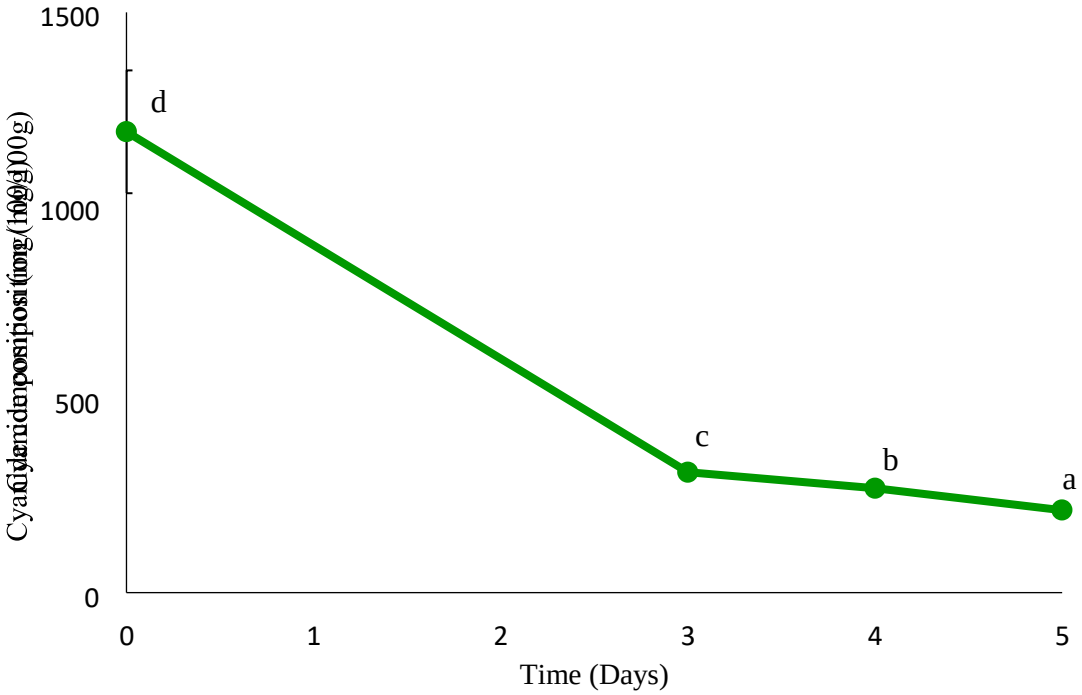


Figure 4.6: Effect of Boiling Time on Cyanide Composition of Cassava Seed Protein Concentrate

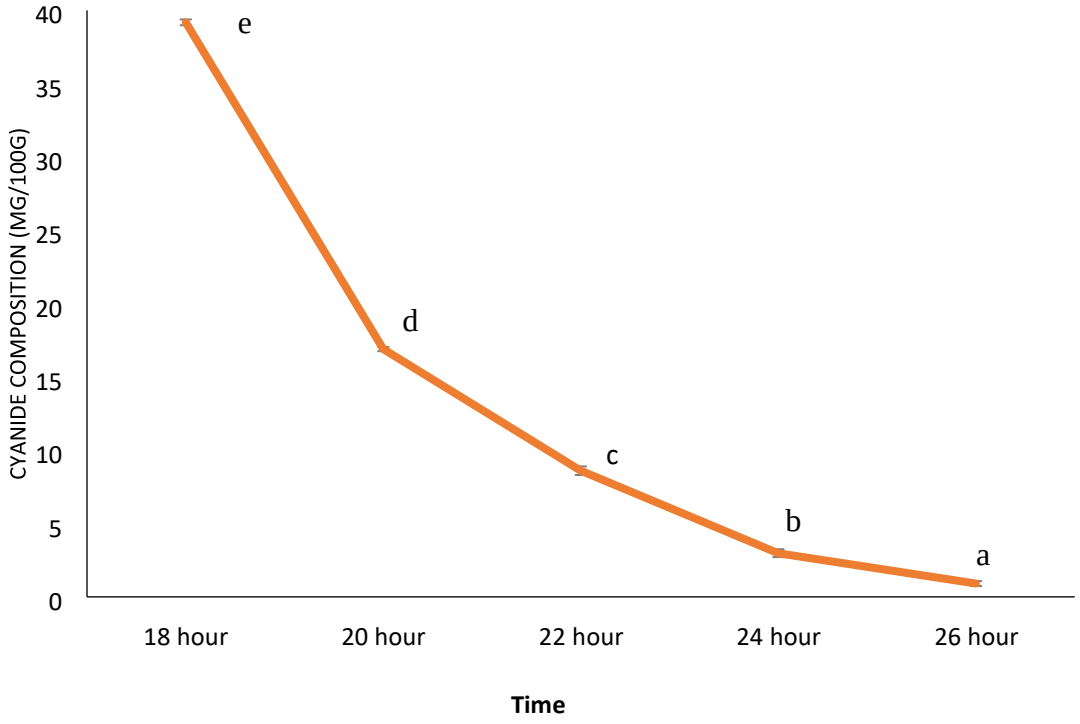
Values are means of triplicate determinations ± SD

Figure 4.7: Effect of Fermentation Time on Cyanide Composition of Cassava Seed Protein Concentrate

1500

4.1.7 Effects of prolonged boiling on cyanide composition of cassava seed protein concentrate

The effect of prolonged boiling for 26 hours on cyanide composition of protein concentrate from cassava seed is shown in Figure 4.8. There was significant decrease ($p<0.05$) in cyanide level as boiling time increased. After 26 hours of boiling, cyanide level was reduced to 0.91 ± 0.15 mg/100g which was lower than the Food and Agriculture Organisation/World Health Organisation (FAO/WHO) tolerable levels of 10 mg/kg (FAO/WHO, 1991).



Values are means of triplicate determinations ± SD

Figure 4.8: Effect of Prolonged Boiling on Cyanide Composition of Cassava Seed Protein Concentrate

4.2 Discussion

4.2.1 Proximate composition of whole and defatted cassava seed flours

Food materials, such as flour, are believed to have lower storage stability than those with moisture contents below 12 % (Twinomuhwezi *et al*., 2020). The lower moisture contents observed in whole cassava seed flour (9.89 ± 0.04 %) (Table 4.1) may increase the shelf stability of cassava seed flour by preventing the growth of microorganisms during storage (Godswill, 2019). The moisture content for whole cassava seed flour obtained in this work is in agreement with that obtained for whole tamarind seed (9.66 ± 0.15 %) by Olagunju *et al*. (2018). The low moisture content obtained in the defatted sample (5.54 ± 1.45 %) may be as a result of the process of defatting (Kumari *et al*., 2018). The moisture content of defatted cassava seed flour (5.54 ± 1.45 %) obtained in this study was lower than 7.68 % reported for defatted sesame seed flour (Lawal *et al*., 2021) which may be because they are of different seeds and environmental factors may also be responsible for the result obtained in this study.

A food material's ash content represents its total mineral content (Pena *et al*., 2020). The ash content of cassava seed in whole flour (4.20 ± 0.23 %) is in agreement with that obtained for whole water melon seeds (4.20 ± 0.02 %) by Ahmad *et al*. (2017). The ash content for defatted flour (7.03 ± 1.08 %) obtained in this study agrees with that for defatted fluted pumpkin seed flour (7.56 ± 0.02) by Alozie *et al*. (2017). There was an increase in the ash content after defatting of the seed flour. Alozie *et al*. (2017) also reported similar increase in ash contents of *Telfairia occidentalis* seed flour after defatting. The high ash content of cassava seed suggests that cassava seed may be a good source of minerals. This suggests that processed cassava seed flour would provide

adequate minerals recommended for animal feed formulation 1.5 – 2.5 % (Fikiru *et al*., 2017).

Fiber is an important component of food. It has been reported to have a significant impact on metabolism in the gastrointestinal tract (Catalkaya *et al*., 2020). Cassava seed flours (whole and defatted) have high crude fibers (11.94 ± 2.10 % and 13.94 ± 1.87 % respectively), which may enhance bowel function and improve faecal bulk digestion. The high crude fibre contents may be due to the presence of the hulls (DePeters *et al*., 2020). The crude fibre levels are in within the range (7.0 - 15.72 %) reported for varieties of orange seed flour and rapeseeds (Akpata and Akubor, 1999; Rahman *et al*., 2019; Carré *et al*., 2016). However, the crude fiber levels are higher than for varieties of legume seed flour (3.09 - 4.50 %) reported by Khattab *et al*. (2009) and lower than that for lima bean flour (18 ± 0.758 %) by Ibeabuchi *et al*. (2019) which could be because they are of different seeds.

Cassava seeds contained appreciable level of oil, suggesting that they can be exploited as source of vegetable oil like the peanuts and soybean seeds. However, the oil content of cassava seed flour (20.75 ± 2.60 %) is lower than (49.13 -56.56 %) reported by Kamuhu *et al*. (2019), OM and Emelike, (2018) and Dravie *et al*. (2020) for groundnut, melon seed and sesame seed flours respectively. However, the oil content in cassava seed flour falls within the range (12.40-21.60 %) of oil contents of conventional oilseed crops (sunflower seed, chia seed and soybean) reported by Adesina (2018), Carrillo *et al*. (2018) and Lisanti and Arwin (2019) respectively.

The crude protein contents (16.64 and 20.75 %) are lower than the range 27.9 - 37.3 % in cassava seed kernels (Nartey and MØller, 1976; Nassar and Dorea, 1982). However, the crude protein content is within the normal range of protein content of legumes (20 – 25 %) reported by Erbersdobler *et al*. (2017) and Maphosa and Jideani, (2017), with exception of soyabeans (37.69 %) reported by Etiosa *et al*. (2017). Although the crude protein content of cassava seed flour (20.75±0.37 %) is at the lower range of protein content of leguminous seeds 20 – 40 % (Sozer *et al*., 2017). Defatting of cassava seed resulted in increase in crude protein content, thus it may potentiate the use of cassava seed flour as source of protein in some food formulations.

Cassava seed flour (whole and defatted) contains appreciable amount of carbohydrate. In fact, carbohydrate was the highest macro nutrient in cassava seed. The carbohydrate level of whole cassava seed flour (35.03 ± 5.73 %) agrees closely with that of whole orange seed flour 34.74 ± 0.01 % (El-Safy *et al*., 2012) but was higher than those for whole chia seed flour 31.46 ± 0.06 % (Carrillo, 2018). Moreover, the carbohydrate level of defatted cassava seed flour 49.99 ± 1.20 % was in close agreement with that of defatted rape seed meal 50.66 % (Jia *et al*., 2021) and for coconut oil cake 49.56 ± 0.99 % (Rodsamran and Sothornvit, 2018). However, the value obtained in this study was lower than those of defatted seed flour of Avocado pear 74.71 ± 1.06 % (Emelike *et al*., 2020), water melon seed 67.6 ± 0.06 % (Gwana *et al.*, 2014) and bambara groundnut seeds 58.1 ± 5.66 (Olaleye *et al*., 2013). Carbohydrate is primarily used as storage form of fuel and structural element. The high carbohydrate content obtained in this study may make it an energy giving source, that offers easily accessible fuel for body functions and physical performance (Hargreaves and Spriet, 2020).

The higher level of ash, crude fibre, crude protein and carbohydrates in the defatted cassava seed compared to the whole seed resulted from the removal of oil from the

defatted samples thereby increasing these parameters. Thus, defatting does not only extract oil for further use, it can increase the content of other nutrients and their potency for food formulation.

4.2.2 Anti-nutrient composition of whole, defatted cassava seed flour and cassava seed protein concentrate

The levels of phytates, tannins, saponins and oxalates are well below the established safe permissible limits in foods (0 – 5 %) (Atiku *et al*., 2016; Ebabhamiegbebho *et al*., 2021). The low levels of phytate and oxalate in this study may indicate that cassava seed flours and concentrate may not interfere with the absorption of mineral elements. The low levels of Saponins and tannins means that the cassava seed flours and concentrate may have no effect on the activity of trypsin and chymotrypsin thereby the digestibility of proteins will be relatively unaffected by the presence of these anti-nutrients (Ebabhamiegbebho *et al*., 2021). The levels of phytates, tannins, saponins and oxalates reported for some protein rich seeds (kidney beans, mung beans and chickpeas) (Samtiya *et al.*, 2020) were higher than those obtained in this study. As a result, cassava seed protein may be safer compared to proteins obtained from these seeds, especially as the level is within the safe permissible limits in foods.

The flavonoid and alkaloid contents were between 2 - 10.5 mg/100g, they are lower than those reported for date palm fruit (34.29 and 5.20 %) for flavonoid and alkaloid (Shaba *et al*., 2015). Cyanide was the highest anti-nutrient; the result obtained for whole cassava seed is slightly higher than 1.17 % for Fabaceae seeds (Nwafor *et al*., 2017). The values obtained for defatted cassava seed and protein concentrate (1141.35 and 1008 mg/100g) are higher than 25.2 mg/kg for sweet almonds (Aranguri-Llerena and Siche, 2020). In this study, the cyanide levels in whole and defatted cassava seed flour are far above the oral

lethal dose of cyanide for humans (10mg/kg) (Aranguri-Llerena and Siche, 2020; Lawrence, 2021). Thus, dietary intake of cassava seed may result in acute or chronic cyanide toxicity, and this might be responsible for its underutilization in the food sector despite its rich nutritional value. Hydrogen cyanide is a known cytochrome oxidase inhibitor and thus interferes with the aerobic respiratory system (Cosmos *et al*., 2020). Therefore, reduction of cyanide to tolerable levels may increase the utilization of cassava seed protein concentrate for industrial purposes.

4.2.3 Functional properties of cassava seed protein concentrate

Bulk density indicates a product's porosity, which influences package design, the amount and strength of packaging material, its texture and mouth feel, and could be used to determine the type of packaging material needed (James *et al.,* 2016; Oloyede *et al*., 2016). In this study, the result obtained (0.75 ± 0.06 g/ml) was low compared to that of varieties of cowpea seed protein concentrate (0.925 g/ml to 1.09 g/ml) but high compared to those of cashew nut protein concentrate (0.31 g/ml) (Ogunwolu *et al*., 2009) and sesame seed protein isolate (0.173 g/ml) (Olasunkanmi *et al*., 2017). Furthermore, the obtained bulk density of cassava seed protein concentrate (CSPC) (0.75 ± 0.06 g/ml) is similar to protein concentrate from bambara groundnut seed cultivars 0.62 – 0.70 g/ml (Adeleke *et al*., 2018). Low bulk density is important in the formulation of complementary foods for infants feeding to improve digestibility because high bulk limits caloric and nutrient intake per feed per child and infants are sometimes unable to consume enough to satisfy their energy and nutrient requirements (Omueti *et al*., 2009; Fasuan *et al*., 2017). Therefore, the low value of bulk density obtained from this study makes the samples suitable for formulation of complementary foods.

The water absorption capacity (WAC) of cassava seed protein concentrate (CSPC)

(250.78 ± 10.22 %) was higher than WAC of protein isolates for legume seeds (97 % - 163 %) (Branch and Maria, 2017; Gunathilake *et al*., 2016; Çelik *et al*., 2019). However, the value obtained is lower than 290 ± 0.04 % for durum wheat bran protein concentrate (Alzuwaid *et al.,* 2020) and 388.04 % for cowpea protein concentrate (Mune *et al.*, 2014). The value obtained in this study is within the range 174.65 ± 0.05 - 311.43 ± 0.25 % for protein concentrate of bambara nut cultivars and chickpea protein concentrates (Ghribi *et al*., 2015; Adeleke *et al*., 2018). The result showed that CSPC, has high values of WAC. This might be due to the presence of polar amino acid residues (Table 4.3), which can increase the affinity of protein concentrates for water molecules, as well as the ability of protein concentrates to swell, dissociate, unfold, and exposing additional binding sites (Zhu *et al*., 2017; Al Maiman *et al*., 2021). Water absorption capacity can be used to determine whether concentrates can be incorporated into aqueous food formulations, particularly those involving dough formulations (Adeleke *et al*., 2018). Water, also helps to improve the mouthfeel and serves as a flavor retainer (Bassogog *et al*., 2020). Therefore, CSPC may be useful in flavor retention, especially those involving soup and dough handling when it is incorporated into aqueous food formulations.

The ability of the protein to absorb oil is an important functionality that influences the taste of fried products. Oil absorption capacity (OAC) of CSPC (209.81 ± 1.77 %) is lower than 270 % – 330 % for rice protein concentrates (Singh and Sogi, 2018) and higher than 130 – 160 % for oat bran protein concentrates (Prosekov *et al*., 2018). The value obtained in this work is in close agreement with that of wheat bran protein concentrate (200 %) (Alzuwaid *et al.,* 2020). The non-polar side chains present in non-polar amino acids (Table 4.3) may bind the hydrocarbon chains of fats, resulting in increased oil absorption (Awuchi *et al*., 2019). Protein with high OAC is important in the formulation of food matrices, such as mayonnaise, sausages, and cake batters (Alzuwaid *et al.,* 2020).

In addition, cassava seed protein concentrate's (CSPC) high water and oil absorption capacity offers it amphipathic properties which would make it a good ingredient in cold meat industry, especially for sausages, where the protein typically bridges the fat and water to produce high- quality products (Jain *et al*., 2019).

Foaming capacity indicates the presence of protein in samples. Which acts as an air or water interface to form stable layer of entrapped air bubbles (Asghari *et al*., 2016). The value obtained in this study 5.5 ± 1.5 % is lower than 24.35 - 40.0 % for defatted walnut flour, walnut protein concentrate and cashew nut protein concentrate at natural pH (Sathe *et al*., 2018). The low foaming capacity was probably due to low solubility, as high protein solubility is required for achieving better foaming capacity (Dhanabalan *et al*. 2020). The foaming capacity of cassava seed protein concentrate obtained in this study would be useful in product formulation requiring low product foaming like dish washing liquids for machines (Toedt *et al*., 2005; Mutungi *et al*., 2019).

Solubility in foods is a chemical and functional property that refers to a food substance's ability to dissolve in a solvent, generally water or oil (Awuchi *et al*., 2019). A protein's solubility is an important functional property that is explored in food systems. Additionally, the solubility of proteins affects a number of functional properties, including emulsification, foaming, and gelation (Kanwate *et al*., 2019). The solubility of CSPC (6.68 ± 0.83 %) is lower than the range (25 - 35.36 %) in cashew nut protein isolate and some legumes flours such as winged bean flour and lablab flour solubility (James *et al*., 2016; Liu *et al*., 2021). However, the value obtained in this study is within the range (1-16 %) for varieties of bean flour concentrate, rice bran protein concentrate, oat bran concentrates and defatted walnut flour concentrate (Sathe *et al*., 2018). Protein solubility is influenced by temperature, ionic strength and pH (Li and Xiong, 2021). The low solubility of CSPC could be due to protein denaturation during fat removal, chemical

treatment during protein extraction, and thermal treatment (James *et al*., 2016). It could also be attributed to the fact that it was extracted at the isoelectric point of the product (Malik and Saini, 2018). Therefore, the pH or other solubility enhancing properties of CSPC can be further modified to enhance its solubility and improved application in the food industry. For example, adding charged amino acids L-Arg and L-Glu to the buffer at 50 mM can greatly increase the solubility of the protein concentrate, these amino acid additives are required to improve protein solubility at high concentrations. In addition to improving protein solubility, these amino acid additives prevent protein aggregation and precipitation and improve sample long-term stability (Kheddo *et al*., 2014).

Least gelling capacity (LGC), a gelation capacity index, is the lowest protein concentration at which gel remained in the inverted tube. The lower the LGC, the better the protein's gelating ability because protein gels are aggregates of denatured molecules (Ajibola *et al*., 2016). The LGC of CSPC (17 ± 1.00 %) was higher than that of varieties of cowpea protein concentrates 12 – 14 % (James *et al*., 2016) but it is in close agreement with that of winged bean flour, wisilate and soy isolate (14 – 18 %) (Sathe *et al*., 2018). Since the LGC of CSPC is not highly different from the LGC of other protein concentrate, the ability of CSPC to form a gel at a higher concentration implies that CSPC have poor gelating ability, hence it will not form a thick gel. This is a good functional property for a complementary diet. This means the diet will be low in dietary bulk. The addition of a thick gel to a complementary diet may have an effect on the child's gastric system, as children have limited gastric capacity to metabolize thick or viscous foods. The importance of this high LGC to the complementary diet is that the diet will have reduced viscosity, plasticity, and elasticity, resulting in a low dietary bulk, which is extremely beneficial for a good complementary diet (Omueti *et al*., 2009). This means that the diet will have low dietary bulk. The addition of a thick gel to a complementary diet may have

an effect on the child's gastric system, as children have limited gastric capacity to metabolize thick or viscous foods. The importance of this high LGC to the complementary diet is that the diet will have reduced viscosity, plasticity, and elasticity, resulting in a low dietary bulk, which is extremely beneficial for a good complementary diet (Omueti *et al*., 2009).

Viscosity is an important food property that influences mouth feel, fluid texture such as beverage, and processing such as pumping, extrusion, and drying (McClements and Grossmann, 2021). The viscosity of CSPC (56.25 ± 4. 65 mpa.s) is in close agreement with that of soy protein 51.2 cp (Shao *et al*., 2016). High viscosity obtained in this study, can be due to the high-water absorption capacity of CSPC as shown in (Table 4.2). Particularly for proteins that exhibit a lesser foamability, the high foaming stability at lower protein concentrations is beneficial from an economic point of view. Studies utilizing a mixture of non-surface-active maltodextrin and low foamability/low foaming stability pea protein isolate showed that the continuous phase's increased viscosity and the protein surface load's influence on foaming stability are both favorable (Moll *et al*., 2020). Therefore, for cassava seed protein concentrate, high viscosity will be necessary for foaming stability, especially as it has low foaming capacity.

4.2.4 Amino acid composition of cassava seed protein concentrate

Amino acid composition of cassava seed protein concentrate (CSCP) is an important chemical property of proteins, as it determines the principal nutritional value of the protein concentrate. Cassava seed protein concentrate contains 18 out of 20 naturally occurring amino acids, therefore, it can be a source of essential and non-essential amino acids (Table 4.5).

The quality of a dietary protein is determined by how well it can supply the essential amino acids needed for tissue upkeep and growth. Since the body is unable to produce essential amino acids, nutrition is required (Filho *et al*., 2017). The total essential amino acids with histidine for CSCP (29.76 g/100g) was found to meet the FAO/WHO (1991) requirements (23.20 g/100g). When comparing the amino acid composition of CSPC with the FAO/WHO (2007) recommended pattern, it appeared that the CSPC essential amino acids concentration are in agreement with the values recommended for a pre-school child (1-2 years old) (Table 4.5), therefore cassava seed protein concentrate contained sufficient amounts of essential amino acids needed to meet the essential amino acid requirement of preschool children. Therefore, the concentrate can be a source of leucine as this amino acid was found to be the highest essential amino acid in the concentrate.

The limiting amino acid in the protein concentrate was found to be lysine with amino acid score of 0.88. This is in agreement with the findings which showed that lysine is generally low in cereal proteins (Rafii *et al*., 2018; Meybodi *et al*., 2019; Leinonen *et al*., 2019) and also, it is the limiting amino acids in most diets (Yin *et al*., 2018). Lysine, in addition to being a protein building block, is a precursor for glutamate, an important signaling amino acid that regulates plant growth and responses to the environment. Lysine levels in plants are regulated by a variety of mechanisms, including intracellular

compartmentalization of enzymes and metabolites, complex transcriptional and posttranscriptional controls of genes encoding enzymes involved in lysine metabolism during plant growth and development, and interactions between different metabolic fluxes (Galili, 2002).

4.2.5 Effects of processing on cyanide levels of cassava seed protein concentrate

Cassava seed has high cyanide content, just like the other parts of the plant. The deleterious effect of this high cyanide level renders processing a pre-requisite for its use. Bolarinwa *et al*. (2016) reported that efficiency of processing depends on the processing technique and the duration of processing.

4.2.5.1 Fermentation

In this study, a time-dependent, significant decrease was observed in the cyanogenic glucoside of cassava seeds during fermentation. Fermentation also results in proportional reduction of hydrocyanic acid to residual amounts in gari samples after 96 hours of processing (Airaodion *et al*., 2019). HCN is decomposed during fermentation, and, as its fermentation time increases, the decomposition of cyanide to the corresponding HCN also increases. In this study, however the long hours of fermentation leading to possible production of alcohol and inability to reduce cyanide to WHO-recommended tolerable levels may limit the utilization of this procedure for cassava seed protein concentrate.

4.2.5.2 Roasting and boiling

Heat processing appears more suitable than fermentation. In this study, the significant decrease ($p<0.05$) observed in the level of cyanogenic glucoside of cassava seed after roasting (Figure 4.5) and boiling (Figure 4.6). This could be due to the effects of high temperature at which cassava seed protein concentrate was roasted and boiled. In fact,

studies have reported that cooking and boiling are among the most effective practices for reducing cyanogenic compounds from food plants (Ndidi *et al*., 2014).

Boiling could be relevant to the elimination of residual cyanide after its release by linamarase, by allowing for translocation and leaching of cell contents including antinutrients and toxic substances (Lemmens *et al*., 2010). In this study, elimination of cyanide in cassava to WHO-recommended levels was achieved through extensive boiling. Previous reports also demonstrated that, boiling of seeds is more efficient over frying in reducing anti-nutrients (Inyang *et al*., 2015). In addition, anti-nutrients in seeds are reduced with higher efficacy and in shorter time through boiling than fermentation (Adeleke *et al*., 2017). Therefore, boiling was considered for further processing.

The heat resulting from high temperature could be responsible for the reduction of the HCN content of the cassava seed. This is in agreement with the observation of Meuser and Smolnik (1980) on the effect of heat on hydro-cyanic acid content. Extensive boiling synergizes the effects of heat treatment and extensive leaching, thereby decreasing cyanide significantly ($p<0.05$) to WHO recommended level of 10 mg/kg (Figure 4.8). The extensive loss of complex structures and extensive time for leaching of the residual cyanide combined could be responsible for the reduction of hydrogen cyanide to tolerable levels after extensive boiling.

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION OF

RESEARCH TO KNOWLEDGE

5.1 Conclusion

Cassava seed contained appreciable amount of nutrients including essential and nonessential amino acids. Apart from cyanide, other antinutrients found in the cassava seed protein concentrate were below the WHO permissible limits.

Cassava seed protein concentrate was found to have high water absorption, oil absorption and swelling capacities which are excellent properties in bakery products and can be incorporated into food matrices.

Traditional processing methods (boiling, roasting and fermentation) were found to progressively reduce the cyanide content in cassava seed protein concentrate but the reduction to innocuous level was only achieved through prolonged boiling. The significant reduction of cyanide during processing suggests that the nutrient in cassava seed protein concentrate will be more bioavailable.

This research work suggests that cassava seed protein concentrate with protein recovery of 84.31 %, when subjected to prolonged boiling can allow for its full potential use as food thereby alleviating protein energy malnutrition in Africa, since it is nutritionally rich in protein.

5.2 Recommendations It

is recommended that:

i. Alternative processing methods like combination of fermentation/roasting, fermentation/boiling, boiling/ roasting and fermentation/roasting should be employed to reduce the cyanide content of cassava seed protein concentrate to innocuous levels.

ii. Biological evaluation (body weight change, net protein utilization, protein digestibility corrected amino acid score and protein efficiency ratio) of the cassava seed protein concentrate be carried out in rats to ascertain the quality of the protein.

5.3 Contribution of Research to Knowledge

This research has established that cassava seed is very rich in protein which when isolated gave a recovery of 84.31 % of protein in the concentrate. The antinutrient of the cassava seed protein concentrate were below WHO permissible limit except for cyanide. The cyanide content was however reduced to innocuous levels by boiling, making the cassava seed protein concentrate a potential good source of protein that could be used as food and food supplement to remedy protein energy malnutrition (PEM).

REFERENCES

Abbas, Y., & Ahmad, A. (2018). Impact of processing on nutritional and anti-nutritional factors of legumes: A review. *Annal of Food Science and Technology*, *19*, 99-215.

Abiodun, O. A., Ayano, B., & Amanyunose, A. A. (2020). Effect of fermentation periods and storage on the chemical and physicochemical properties of biofortified cassava gari. *Journal of Food Processing and Preservation*, *44*(12), e14958.

Abraham, K., Buhrke, T., & Lampen, A. (2016). Bioavailability of cyanide after consumption of a single meal of foods containing high levels of cyanogenic glycosides: a crossover study in humans. *Archives of Toxicology*, *90*(3), 559-574.

Achidi, A. U., Ajayi, O. A., Maziya-dixon, B. U. S. S. I. E., & Bokanga, M. (2008). The effect of processing on the nutrient content of cassava (Manihot esculenta Crantz) leaves. *Journal of Food Processing and Preservation*, *32*(3), 486-502.

Adamu, A., Adie, D. B., & Alka, U. A. (2014). A comparative study of the use of cassava species and alum in waste water treatment. *Nigerian Journal of Technology*, *33*(2), 170-175.

Adedokun, O. O. (2019). Carcass Characteristics, Organ Proportion and Economics of Broiler Chickens Fed Graded Levels of Processed *Manihot esculentum crantz* (var. Umucass 36) Cassava Composite Meal. *Nigerian Journal of Animal Science and Technology*, *2*(3), 46-54.

Adegbenro, M. (2018). Evaluation of the nutrients and some bioactive components in some under-utilized seeds. *Archiva Zootechnica*, *21*(2), 49-58.

Adeleke, B. S., & Babalola, O. O. (2020). Oilseed crop sunflower (Helianthus annuus) as a source of food: Nutritional and health benefits. *Food Science & Nutrition*, *8*(9), 4666-4684.

Adeleke, O. R., Adiamo, O. Q., & Fawale, O. S. (2018). Nutritional, physicochemical, and functional properties of protein concentrate and isolate of newly-developed Bambara groundnut (*Vigna subterrenea* L.) cultivars. *Food Science and Nutrition*, *6*(1), 229-242.

Adeleke, O., Adiamo, O. Q., Fawale, O. S., & Olamiti, G. (2017). Effect of soaking and boiling on anti-nutritional factors, oligosaccharide contents and protein digestibility of newly developed bambara groundnut cultivars. *Turkish Journal of Agriculture-Food Science and Technology*, *5*(9), 1006-1014.

Adenekan, M. K., Fadimu, G. J., Odunmbaku, L. A., Nupo, S. S., Oguntoyinbo, S. I., & Oke, E. K. (2017). Chemical and functional characterization of baobab (*Adansonia digitata* L.) seed protein concentrate using alcohol extraction method. *International Journal of Environment, Agriculture and Biotechnology*, *2*(5), 238938-239936.

Adepoju, A. A., Oladeebo, J. O., & Toromade, A. S. (2019). Analysis of occupational hazards and poverty profile among cassava processors in Oyo State, Nigeria. *Asian Journal of Advances in Agricultural Research*, *9*(1), 1-13.

Adesina, S. A. (2018). Effect of processing on the proximate composition of sunflower (*Helianthus annuus*) seeds. *Agro-Science*, *17*(3), 27-33.

Adiele, J. G., Schut, A. G. T., van den Beuken, R. P. M., Ezui, K. S., Pypers, P., Ano, A. O., & Giller, K. E. (2021). A recalibrated and tested LINTUL-Cassava simulation model provides insight into the high yield potential of cassava under rainfed conditions. *European Journal of Agronomy*, *124*, 126242-126253.

Agnoli, C., Baroni, L., Bertini, I., Ciappellano, S., Fabbri, A., Papa, M., & Sieri, S. (2017). Position paper on vegetarian diets from the working group of the Italian Society of Human Nutrition. *Nutrition, Metabolism and Cardiovascular*

Diseases, *27*(12), 1037-1052.

Ahmad, M., Faruk, R., Shagari, K. A., & Umar, S. (2017). Analysis of Essential Oil from Watermelon Seeds. *SosPoly Journal of Science and Agriculture*, *2*, 1–17.

Ahmed, S., Ahmed, N., Rungatscher, A., Linardi, D., Kulsoom, B., Innamorati, G., & Faggian, G. (2020). Cocoa flavonoids reduce inflammation and oxidative stress in a myocardial ischemia-reperfusion experimental model. *Antioxidants*, *9*(2), 167-186.

Airaodion, A. I., Airaodion, E. O., Ewa, O., Ogbuagu, E. O., & Ogbuagu, U. (2019). Nutritional and anti–nutritional evaluation of garri processed by traditional and instant mechanical methods. *Asian Food Science Journal*, *9*(4), 1-13.

Airaodion, A. I., & Ogbuagu, E. O. (2020). Effect of consumption of garri processed by traditional and instant mechanical methods on lipid profile of Wistar rats. *Asian Journal of Research and Reports in Gastroenterology*, *3*(1), 26-33.

Ajibola, C. F., Malomo, S. A., Fagbemi, T. N., & Aluko, R. E. (2016). Polypeptide composition and functional properties of African yam bean seed (*Sphenostylis stenocarpa*) albumin, globulin and protein concentrate. *Food Hydrocolloids*, *56*, 189-200.

Ajibola, G. O., & Olapade, A. A. (2016). Physical, proximate and anti-nutritional composition of African yam bean (*Sphenostylis stenocarpa*) seeds varieties. *Journal of Food Research*, *5*(2), 67-70.

Akinboye, A. J., Kim, K., Choi, S., Yang, I., & Lee, J. G. (2023). Alkaloids in food: a review of toxicity, analytical methods, occurrence and risk assessments. *Food Science and Biotechnology*, *32*(9), 1133-1158.

Akinsanmi, S. K., Igbasan, F. A., Agbede, J. O., & Akinnusotu, A. (2020). Growth performance and Carcass Characteristics of broiler Chickens fed Graded levels of differently processed Rubber Seed Meal based Diets. *International Journal of Environmental, Agriculture and Biotechnology*, *5*(4), 1187-1195.

Akomolafe, J. K., Sennuga, S. O., Bamidele, J., Osho-Lagunju, B., & Alabuja, F. O. (2023). Assessment of Cassava Production towards Household Food Security in Bwari Area Council, Abuja, Nigeria. *Indiana Journal of Agriculture and Life Sciences*, *3*(2), 1-7.

Akpata, M. I., & Akubor, P. I. (1999). Chemical composition and selected functional properties of sweet orange (*Citrus sinensis*) seed flour. *Plant Foods for Human Nutrition*, *54*(4), 353-362.

Al Hasan, S. M., Hassan, M., Saha, S., Islam, M., Billah, M., & Islam, S. (2016). Dietary phytate intake inhibits the bioavailability of iron and calcium in the diets of pregnant women in rural Bangladesh: a cross-sectional study. *BMC Nutrition*, *2*(1), 1-10.

Al Maiman, S. A., bin Ali Al-qahtani, M., Alfawaz, M. A., Albader, N. A., Alsualeem, S., Osman, M. A., & Hassan, A. B. (2021). Protein profile and functional properties of defatted flour, protein concentrate, and isolate of Al-Samh (*Mesembryanthemum forsskalei Hochst*) seeds. *Journal of the Saudi Society for Food and Nutrition, 14*(1), 12-23.

Alafid, F., Edrah, S. M., Meelad, F. M., Belhaj, S., Altwair, K., & Maizah, N. R. (2019). Evaluation of phytochemical constituents and antibacterial activity of *thymelaea hirsuta* (l.) Endl, and that utilised as a conventional treatment of infertility and diabetic in Libya. *World Journal of Pharmaceutical Research, 8*(11), 72-88.

Alamu, E. O., Prisca, C., Olaniyan, B., Omosebi, M. O., Adegunwa, M. O., Chikoye, D., & Maziya-Dixon, B. (2021). Evaluation of nutritional properties, and consumer preferences of legume-fortified cassava leaves for low-income households in Zambia. *Cogent Food and Agriculture*, *7*(1), 1885796- 2764485.

Alemu, D., Rashid, S., & Tripp, R. (2019). Seed system potential in Ethiopia: Constraints and opportunities for enhancing the seed sector. *Gates Open Research 3*(948), 948-954.

Alexis, T. F. C., Pelagie, Y., Franck, Y., & Valentin, W. (2020). Nutritional Contributions to the Physical-Chemical and Biochemical Properties of Cassava (Manihot Esculenta Crantz) Leaves Grown in South Benin. *Chemical Science International Journal*, *29*(8), 1-6.

Alford, D.V. (2007). *Pests of Fruit Crop: A Colour Handbook* (pp. 19-138). Burlington, MA, USA: Academic Press.

Ali, A. S., & Elozeiri, A. A. (2017). Metabolic processes during seed germination. *Advances in Seed Biology, 2017*, 141-166.

Allem, A. C. (1994). The origin of Manihot esculenta crantz (Euphorbiaceae). *Genetic Resources and Crop Evolution*, *41*, 133-150.

Alozie, Y., Udo, A., & Orisa, C. (2017). Proximate, anti-nutrient and vitamin composition of full-fat and defatted seed flour of *Telfairia occidentalis*. *Turkish Journal of Agriculture-Food Science and Technology*, *5*(11), 1256-1260.

Alves, A. A. C. (2001). Cassava botany and physiology. *Cassava: Biology, Production and Utilization* (pp. 67-89). Wallingford UK: Cabi Publishing.

Alves, A. A. C., Manthey, L., Isbell, T., Ellis, D., & Jenderek, M. M. (2014). Diversity in oil content and fatty acid profile in seeds of wild cassava germplasm. *Industrial Crops and Products*, *60*, 310-315.

Alves-Pereira, A., Clement, C. R., Picanço-Rodrigues, D., Veasey, E. A., Dequigiovanni, G., Ramos, S. L., & Zucchi, M. I. (2018). Patterns of nuclear and chloroplast genetic diversity and structure of manioc along major Brazilian Amazonian rivers. *Annals of Botany, 121*(4), 625-639.

Alzuwaid, N. T., Sissons, M., Laddomada, B., & Fellows, C. M. (2020). Nutritional and functional properties of durum wheat bran protein concentrate. *Cereal Chemistry*, *97*(2), 304-315.

Amala, B. (2016). Studies on Development and Standardization of Multigrain Bar enriched with Glaxseed by using Stevia for Anti-diabetic Purpose (Doctoral dissertation, Ph. D. Thesis, college of food technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, India).

Amza, T., Amadou, I., Kamara, M. T., Zhu, K. X., & Zhou, H. M. (2011). Nutritional and functional characteristics of gingerbread plum (*Neocarya macrophylla*): an underutilized oilseed. *Grasas y Aceites*, *62*(3), 290-298.

Analin, B., Mohanan, A., Bakka, K., & Challabathula, D. (2020). Cytochrome oxidase and alternative oxidase pathways of mitochondrial electron transport chain are important for the photosynthetic performance of pea plants under salinity stress conditions. *Plant Physiology and Biochemistry*, *154*, 248-259.

Ao, C. (2019). The endosperm development and the variations of structures of embryo sacs: unravelling the low fecundity of Zephyranthes candida (Amaryllidaceae). *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *153*(5), 673-678.

AOAC (2012). International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces. *AOAC Official Methods of Analysis*.

AOAC (2005). *Official methods of the Association of Official Analytical Chemists*, 18th edition. Washington, D. C.

Aranguri-Llerena, G., & Siche, R. (2020). Superior plants with significant amounts of cyanide and their toxicological implications. *Reviews in Agricultural Science*, *8*, 354-366.

Aravani, V. P., Sun, H., Yang, Z., Liu, G., Wang, W., Anagnostopoulos, G., & Papadakis, V. G. (2022). Agricultural and livestock sector's residues in Greece and China: comparative qualitative and quantitative characterization for assessing their potential for biogas production. *Renewable and Sustainable Energy Reviews*, *154*, 111821-1216111.

Arbab Sakandar, H., Chen, Y., Peng, C., Chen, X., Imran, M., & Zhang, H. (2023). Impact of fermentation on antinutritional factors and protein degradation of legume seeds: A review. *Food Reviews International*, *39*(3), 1227-1249.

Aremu, M., Ibrahim, H., & Ekanem, B. (2016). Effect of processing on in–vitro protein digestibility and anti–nutritional properties of three underutilized legumes grown in Nigeria. *British Biotechnology Journal*, *14*(1), 1-10.

Arrutia, F., Binner, E., Williams, P., & Waldron, K. W. (2020). Oilseeds beyond oil: Press cakes and meals supplying global protein requirements. *Trends in Food Science and Technology*, *100*, 88-102.

Arshad, N., Akhtar, S., Ismail, T., Saeed, W., Qamar, M., Özogul, F., & Rocha, J. M. (2023). The Comparative Effect of Lactic Acid Fermentation and Germination on the Levels of Neurotoxin, Anti-Nutrients, and Nutritional Attributes of Sweet Blue Pea (*Lathyrus sativus* L.). *Foods*, *12*(15), 2851-2856.

Asghari, A. K., Norton, I., Mills, T., Sadd, P., & Spyropoulos, F. (2016). Interfacial and foaming characterisation of mixed protein-starch particle systems for food-foam applications. *Food Hydrocolloids*, *53*, 311-319.

Atiku, A., Oladipo, O. O., Forcados, G. E., Usman, A. S., & Mancha, M. D. (2016). Antinutritional and phytochemical profile of some plants grazed upon by ruminants in North Central Nigeria during the dry season (January to April). *International Journal of Livestock Production*, *7*(4), 19-23.

Atobrah, E. E. (2020). Orange-fleshed Sweet Potato (ofsp)–Cassava Composite Gari: Effects of Processing Variables and Storage on Beta-carotene and Sensory Qualities (Doctoral dissertation, University of Cape Coast).

Atrahimovich, D., Avni, D., & Khatib, S. (2021). Flavonoids-Macromolecules Interactions in Human Diseases with Focus on Alzheimer, Atherosclerosis and Cancer. *Antioxidants*, *10*(3), 423-433.

Awuchi, C. G., Igwe, V. S., & Echeta, C. K. (2019). The functional properties of foods and flours. *International Journal of Advanced Academic Research*, *5*(11), 139160.

Bareke, T. (2018). Biology of seed development and germination physiology. *Advances in Plants and Agriculture Research*, *8*(4), 336-46.

Baroux, C., & Grossniklaus, U. (2019). Seeds—An evolutionary innovation underlying reproductive success in flowering plants. *Current Topics in Developmental Biology*, *131*, 605-642.

Bartges, J. W. (2016). Feline calcium oxalate urolithiasis: risk factors and rational treatment approaches. *Journal of Feline Medicine and Surgery*, *18*(9), 712-722.

Bassi, D., Kaur, K., Singh, T. P., & Kaur, J. (2021). Quality attributes and Chapatti making property of biofortified wheat as influenced by particle size. *Journal of Food Science and Technology*, *58*(3), 1156-1164.

Bassogog, C. B., Bakepeck, P., Nyobe, C., Panyo'o, E., Okella, E., & Edoun, F. (2020). Chemical composition, antioxidant, alpha-Amylase inhibitory and functional properties of *Cucumeropsis mannii* seeds protein concentrate. *Journal of Food Processing and Technology*, *11*, 826- 827.

Bayata, A. (2019). Review on nutritional value of cassava for use as a staple food. *Science Journal of Analytical Chemistry*, *7*(4), 83-91.

Bechoff, A., Tomlins, K., Fliedel, G., Becerra Lopez-Lavalle, L. A., Westby, A., Hershey, C., & Dufour, D. (2018). Cassava traits and end-user preference: Relating traits to consumer liking, sensory perception, and genetics. *Critical Reviews in Food Science and Nutrition*, *58*(4), 547-567.

Bhadouria, J., Singh, A. P., Mehra, P., Verma, L., Srivastawa, R., Parida, S. K., & Giri, J. (2017). Identification of purple acid phosphatases in chickpea and potential roles of CaPAP7 in seed phytate accumulation. *Scientific Reports*, *7*(1), 1-12.

Bharadwaj, M., Lata, M., & Mondal, B. C. (2021). Scope of utilization of tannin & saponin to improve animal performance. *~ 2168 ~ Journal of Entomology and Zoology Studies*, *9*(1), 2168–2179.

Bhattarai, S., Coulman, B., & Biligetu, B. (2016). Sainfoin (*Onobrychis viciifolia Scop*.): renewed interest as a forage legume for western Canada. *Canadian Journal of Plant Science*, *96*(5), 748-756.

Biglar, M., Moghaddam, G., Sadeghi, N., Oveisi, M. R., Jannat, B., Kaboli, Z., & Hajimahmoodi, M. (2012). Profiling of major fatty acids in different raw and roasted sesame seeds cultivars. *African Journal of Biotechnology*, *11*(24), 66196623.

Boakye Peprah, B., Parkes, E. Y., Harrison, O. A., van Biljon, A., Steiner-Asiedu, M., & Labuschagne, M. T. (2020). Proximate composition, cyanide content, and carotenoid retention after boiling of provitamin A-rich cassava grown in Ghana. *Foods*, *9*(12), 1800-1900.

Bolarinwa, I. F., Olaniyan, S. A., Olatunde, S. J., Ayandokun, F. T., & Olaifa, I. A. (2016). Effect of processing on amygdalin and cyanide contents of some Nigerian foods. *Journal of Chemical and Pharmaceutical Research*, *8*(2), 106-113.

Bolhuis, G. G. (1954). The toxicity of cassava roots. *Netherlands Journal of Agricultural Science*, *2*(3), 176-185.

Bonanno, J. A. (2020). Toxicokinetics of Cyanide and Its Metabolites, and Related Gene Expression in Marine Fish: Implications towards Combating Cyanide Fishing through Detection (Doctoral dissertation, University of Massachusetts Boston).

Bong, W. C., Vanhanen, L. P., & Savage, G. P. (2017). Addition of calcium compounds to reduce soluble oxalate in a high oxalate food system. *Food Chemistry*, *221*, 5457.

Bouajila, A., Ammar, H., Chahine, M., Khouja, M., Hamdi, Z., Khechini, J., & López, S. (2020). Changes in phytase activity, phosphorus and phytate contents during grain germination of barley (*Hordeum vulgare* L.) cultivars. *Agroforestry Systems*, 94(4), 1151-1159.

Bradbury, E. J., Duputié, A., Delêtre, M., Roullier, C., Narváez-Trujillo, A., ManuAduening, J. A., & McKey, D. (2013). Geographic differences in patterns of genetic differentiation among bitter and sweet manioc (Manihot esculenta subsp. esculenta; Euphorbiaceae). *American Journal of Botany*, *100*(5), 857-866.

Branch, S., & Maria, S. (2017). Evaluation of the functional properties of mung bean protein isolate for development of textured vegetable protein. *International Food Research Journal*, *24*(4), 1595-1605.

Breuninger, W. F., Piyachomkwan, K., & Sriroth, K. (2009). Tapioca/Cassava Starch: Production and Use, 3rd edition. In *Starch* (pp. 541–568). Burlington, MA, USA: Academic Press.

Brzezicha-Cirocka, J., Grembecka, M., & Szefer, P. (2016). Oxalate, magnesium and calcium content in selected kinds of tea: impact on human health. *European Food Research and Technology*, *242*(3), 383-389.

Bulkan, J. (2019). The place of bitter cassava in the social organization and belief systems of two indigenous peoples of Guyana. *Culture, Agriculture, Food and Environment*, *41*(2), 117-128.

Byju, G., & Suja, G. (2020). Mineral nutrition of cassava. *Advances in Agronomy*, *159*, 169-235.

Carré, P., Citeau, M., Robin, G., & Estorges, M. (2016). Hull content and chemical composition of whole seeds, hulls and germs in cultivars of rapeseed (*Brassica napus*). *Oilseeds and Fats, Crops and Lipids*, *23*(3), A302.

Carrillo, W., Cardenas, M., Carpio, C., Morales, D., Álvarez, M., & Silva, M. (2018). Content of nutrients component and fatty acids in chia seeds (*Salvia hispanica* L.) cultivated in Ecuador. *Asian Journal of Pharmaceutical and Clinical Research*, 11, 1-4.

Catalkaya, G., Venema, K., Lucini, L., Rocchetti, G., Delmas, D., Daglia, M., & Capanoglu, E. (2020). Interaction of dietary polyphenols and gut microbiota: Microbial metabolism of polyphenols, influence on the gut microbiota, and implications on host health. *Food Frontiers*, *1*(2), 109-133.

Ceballos, H., & Hershey, C. H. (2017). Cassava (*Manihot esculenta Crantz*). *In Genetic Improvement of Tropical Crops* (pp. 129-180). Cham, Switzerland: Springer International Publishing.

Ceballos, H., De la Cruz, A., & Gabriel, A. (2012). Cassava taxonomy and morphology. *Cassava in the Third Millenium modern production,processing,use and marketing systems* (pp. 15–28). International Centre for Tropical Agriculture (CIAT), Colombia: Cali.

Çelik, M., Güzel, M., & Yildirim, M. (2019). Effect of pH on protein extraction from sour cherry kernels and functional properties of resulting protein concentrate. *Journal of Food Science and Technology*, *56*(6), 3023-3032.

Chagas, F. O., de Cassia Pessotti, R., Caraballo-Rodriguez, A. M., & Pupo, M. T. (2018). Chemical signaling involved in plant–microbe interactions. *Chemical Society Reviews*, *47*(5), 1652-1704.

Chandi, G.K. & Sogi, D.S. (2007). Functional Properties of Rice Bran Protein Concentrates. *Journal of Food Engineering*, *79*, 592-597.

Chatterjee, C., Gleddie, S., & Xiao, C. W. (2018). Soybean bioactive peptides and their functional properties. *Nutrients*, 10(9), 1211-1345.

Chen, K. I., Chiang, C. Y., Ko, C. Y., Huang, H. Y., & Cheng, K. C. (2018). Reduction of phytic acid in soymilk by immobilized phytase system. *Journal of Food Science*, *83*(12), 2963-2969.

Cheng, D., Li, D., Yuan, Y., Zhou, L., Li, X., Wu, T., & Sun, Y. (2017). Improving carbohydrate and starch accumulation in Chlorella sp. AE10 by a novel two-stage process with cell dilution. *Biotechnology for Biofuels*, *10*(1), 1-14.

Chongtham, N., Bisht, M. S., Premlata, T., Bajwa, H. K., Sharma, V., & Santosh, O. (2021). Quality improvement of bamboo shoots by removal of antinutrients using different processing techniques: A review. *Journal of Food Science and Technology*, *59*, 1-11.

Conklin, P. A., Strable, J., Li, S., & Scanlon, M. J. (2019). On the mechanisms of development in monocot and eudicot leaves. *New Phytologist*, *221*(2), 706-724.

Cosman, F., Crittenden, D. B., Adachi, J. D., Binkley, N., Czerwinski, E., Ferrari, S., & Grauer, A. (2016). Romosozumab treatment in postmenopausal women with osteoporosis. *New England Journal of Medicine*, *375*(16), 1532-1543.

Cosmos, A., Erdenekhuyag, B. O., Yao, G., Li, H., Zhao, J., Laijun, W., & Lyu, X. (2020). Principles and methods of bio detoxification of cyanide contaminants. *Journal of Material Cycles and Waste Management*, *22*, 939-954.

Coulibaly, A., Kouakou, B., & Chen, J. (2011). Phytic acid in cereal grains: Structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. *American Journal of Plant Nutrition and Fertilization Technology*, *1*(1), 1–22.

Cressey, P., & Reeve, J. (2019). Metabolism of cyanogenic glycosides: A review. *Food and Chemical Toxicology*, *125*, 225-232.

Danne, R., Poojari, C., Martinez-Seara, H., Rissanen, S., Lolicato, F., Róg, T., & Vattulainen, I. (2017). doGlycans–tools for preparing carbohydrate structures for atomistic simulations of glycoproteins, glycolipids, and carbohydrate polymers for GROMACS. *Journal of Chemical Information and Modeling*, *57*(10), 24012406.

De Bruyn, A., Harimalala, M., Zinga, I., Mabvakure, B. M., Hoareau, M., Ravigné, V., & Lefeuvre, P. (2016). Divergent evolutionary and epidemiological dynamics of cassava mosaic geminiviruses in Madagascar. *BioMed Central Evolutionary Biology*, *16*(1), 1-21.

de Camargo, A. C., & da Silva Lima, R. (2019). A perspective on phenolic compounds, their potential health benefits, and international regulations: The revised Brazilian normative on food supplements. *Journal of Food Bioactives*, *7*, 7–17.

de Camargo, A. C., Favero, B. T., Morzelle, M. C., Franchin, M., Alvarez-Parrilla, E., de la Rosa, L. A., & Schwember, A. R. (2019). Is chickpea a potential substitute for soybean? Phenolic bioactives and potential health benefits. *International Journal of Molecular Sciences*, *20*(11), 2644-2766.

De Girolamo, A., Lippolis, V., & Pascale, M. (2022). Overview of recent liquid chromatography mass spectrometry-based methods for natural toxins detection in food products. *Toxins, 14*(5), 328-330.

De Jesus, J. H. F., Ferreira, A. P. G., & Cavalheiro, E. T. G. (2018). Thermal behavior of inosine 5′-monophosphate in acidic form and as alkali and alkaline earth salts. *Food Chemistry, 258*, 199-205.

De Souza, A. P., Massenburg, L. N., Jaiswal, D., Cheng, S., Shekar, R., & Long, S. P. (2017). Rooting for cassava: insights into photosynthesis and associated physiology as a route to improve yield potential. *New Phytologist, 213*(1), 50-65.

Del Hierro, J. N., Casado-Hidalgo, G., Reglero, G., & Martin, D. (2021). The hydrolysis of saponin-rich extracts from fenugreek and quinoa improves their pancreatic lipase inhibitory activity and hypocholesterolemic effect. *Food Chemistry, 338*, 128113-212211.

Delaquis, E., Andersen, K. F., Minato, N., Cu, T. T. L., Karssenberg, M. E., Sok, S., & de Haan, S. (2018). Raising the stakes: cassava seed networks at multiple scales in Cambodia and Vietnam. *Frontiers in Sustainable Food Systems, 2*, 73-76.

Delêtre, M., Lett, J. M., Sulpice, R., & Spillane, C. (2021). Kinship networks of seed exchange shape spatial patterns of plant virus diversity. *Nature Communications, 12*(1), 4505-5625.

Deng, P., Cui, B., Zhu, H., Phommakoun, B., Zhang, D., Li, Y., & Zhao, Z. (2021). Accumulation Pattern of Amygdalin and Prunasin and Its Correlation with Fruit and Kernel Agronomic Characteristics during Apricot (*Prunus armeniaca* L.) Kernel Development. *Foods, 10*(2), 397-379.

DePeters, E. J., Swanson, K. L., Bill, H. M., Asmus, J., & Heguy, J. M. (2020). Nutritional composition of almond hulls. *Applied Animal Science, 36*(6), 761-770.

D'Este, M., Alvarado-Morales, M., & Angelidaki, I. (2018). Amino acids production focusing on fermentation technologies–A review. *Biotechnology Advances, 36*(1), 14-25.

Dhanabalan, V., Xavier, M., Murthy, L. N., Asha, K. K., Balange, A. K., & Nayak, B. B. (2020). Evaluation of physicochemical and functional properties of spray-dried protein hydrolysate from non-penaeid shrimp (*Acetes indicus*). *Journal of the Science of Food and Agriculture, 100*(1), 50-58.

Díaz-Mula, H. M., Tomás-Barberán, F. A., & García-Villalba, R. (2019). Pomegranate fruit and juice (cv. Mollar), rich in ellagitannins and anthocyanins, also provide a significant content of a wide range of proanthocyanidins. *Journal of Agricultural and Food Chemistry, 67*(33), 9160-9167.

Diouf, A., Sarr, F., Sene, B., Ndiaye, C., Fall, S. M., & Ayessou, N. C. (2019). Pathways for reducing anti-nutritional factors: Prospects for *Vigna unguiculata*. *Journal of Nutritional Health and Food Science, 7*(2), 1-10.

Dravie, E., Nii, K., Edward, K., Clement, O., Adjoa, A., & Gaston, H. (2020). "Antioxidant, phytochemical and physicochemical properties of sesame seed (*Sesamum indicum* L.). *Scientific African,* 8, e00349.

Dufour, D. L., Piperata, B. A., Murrieta, R. S., Wilson, W. M., & Williams, D. D. (2016). Amazonian foods and implications for human biology. *Annals of Human Biology*, *43*(4), 330-348.

Ebabhamiegbebho, P. A., Olapade, A. A., Obomeghei, A. A., & Oguntoye, I. L. (2021). Evaluation of the vitamins, minerals, and phytochemical contents of complementary food blends from pearl millet (*pennisetum glaucum*), African yam bean (*sphenostylis stenocarpa* hoechst ex. a. rich), and tiger nut (*cyperus esculentus*). *Medico Research Chronicles*, *8*(3), 205-215.

Edhirej, A., Sapuan, S. M., Jawaid, M., & Zahari, N. I. (2017). Cassava: Its polymer, fiber, composite, and application. *Polymer Composites*, *38*(3), 555-570.

Ehsen, S., Qasim, M., Abideen, Z., Rizvi, R. F., Gul, B., Ansari, R., & Khan, M. A. (2016). Secondary metabolites as anti-nutritional factors in locally used halophytic forage/fodder. *Pakistan Journal of Botany*, *48*(2), 629-636.

Ekop, M. (2020). Tracking Cyanogenic Potentials of Cassava Tuber Processing into "GARI" from Farm to Table and Assessing Toxicity Levels. *Journal of Food Chemistry and Nanotechnology*, *6*(1), 33-39.

Elkonin, L. A., Italianskaya, J. V., Domanina, I. V., Selivanov, N. Y., Rakitin, A. L., & Ravin, N. V. (2016). Transgenic sorghum with improved digestibility of storage proteins obtained by Agrobacterium-mediated transformation. *Russian Journal of Plant Physiology*, *63*(5), 678-689.

El-Safy, F. S., Salem, R. H., & Abd El-Ghany, M. E. (2012). Chemical and nutritional evaluation of different seed flours as novel sources of protein. *World Journal of Dairy and Food Sciences*, *7*(1), 59-65.

Emelike, N. J. T., Ujong, A. E., & Achinewhu, S. C. (2020). Proximate composition, mineral bioavailability and functional properties of defatted and undefatted avocado pear (*Persia americana*) seed flours. *Asian Food Science Journal, 17*(3), 1-10.

Enujiugha, V. N. (2017). Improving agricultural productivity through increased local biodiversity exploitation and food composition database management. *Journal of Biodiversity, Bioprospecting and Development*, *4*(1), 162-164.

Erbersdobler, H. F., Barth, C. A., & Jahreis, G. (2017). Legumes in human nutrition. Nutrient content and protein quality of pulses. *Ernahrungs Umschau*, *64*(9), 134139.

Erhan, S. Z., & Adhvaryu, A. (2020). Non-food lipids. In *Plant lipids* (pp. 103-122). Oxford UK: Blackwell Publishing Ltd.

Ertop, M. H., & Bektaş, M. (2018). Enhancement of bioavailable micronutrients and reduction of antinutrients in foods with some processes. *Food and Health*, *4*(3), 159–165.

Estupiñan-López, L., Campos, V. P., Silva, A. P., Barros, A. F., Pedroso, M. P., Silva, J. C., & Terra, W. C. (2017). Volatile organic compounds from cottonseed meal are toxic to Meloidogyne incognita. *Tropical Plant Pathology*, *42*(6), 443-450.

Esuma, W., Kawuki, R. S., Herselman, L., & Labuschagne, M. T. (2016). Diallel analysis of provitamin A carotenoid and dry matter content in cassava (*Manihot esculenta Crantz*). *Breeding science*, *66*(4), 627-635.

Etiosa, O., Chika, N., & Benedicta, A. (2018). Mineral and proximate composition of soya bean. *Asian Journal of Physical and Chemical Sciences*, *4*(3), 1-6.

Ezui, K. S., Franke, A. C., Mando, A., Ahiabor, B. D. K., Tetteh, F. M., Sogbedji, J., & Giller, K. E. (2016). Fertiliser requirements for balanced nutrition of cassava across eight locations in West Africa. *Field Crops Research*, *185*, 69-78.

Fan, R., Hong, X., Min, H., Yi, Q., Zhang, Q., & Luo, Z. (2019). Preparation of formaldehyde-free persimmon tannin-based rigid foam as an adsorbent for selective recovery of Au (III) ions. *Desalination and Water Treatment*, *151*, 219229.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). (2007). Protein and amino acid requirements in human nutrition: Report of a joint FAO/WHO expert consultation. *World Health Organisation Technical Report Series*, *935*, 1-265.

FAO/WHO. (1991). Protein quality evaluation; Report of the joint FAO/WHO expert consultation; food and nutrition paper. Rome, Italy. *Food and Agriculture Organization of the United Nation*, *51*.

Fasuan, T., Fawale, S. O., Enwerem, D. E., Uche, N., & Ayodele, E. A. (2017). Physicochemical, functional and economic analysis of complementary food from cereal, oilseed and animal polypeptide. *International Food Research Journal*, *24*(1), 275–283.

Fasuyi, A. O. (2005). Nutrient composition and processing effects on cassava leaf (*Manihot esculenta Crantz*) antinutrients. *Pakistan Journal of Nutrition*, *4*(1), 3742.

Fenn, M. A., & Giovannoni, J. J. (2021). Phytohormones in fruit development and maturation. *The Plant Journal*, *105*(2), 446-458.

Ferguson, M. E., Shah, T., Kulakow, P., & Ceballos, H. (2019). A global overview of cassava genetic diversity. *PloS One*, *14*(11), e0224763.

Ferraro, V., Piccirillo, C., Tomlins, K., & Pintado, M. E. (2016). Cassava (Manihot esculenta Crantz) and yam (Dioscorea spp.) crops and their derived foodstuffs:

safety, security and nutritional value. *Critical Reviews in Food Science and Nutrition*, *56*(16), 2714-2727.

Fikiru, O., Bultosa, G., Fikreyesus Forsido, S., & Temesgen, M. (2017). Nutritional quality and sensory acceptability of complementary food blended from maize (*Zea mays*), roasted pea (*Pisum sativum*), and malted barley (*Hordium vulgare*). *Food Science and Nutrition*, *5*(2), 173-181.

Filho, A. M. M., Pirozi, M. R., Borges, J. T. D. S., Pinheiro Sant'Ana, H. M., Chaves, J. B. P., & Coimbra, J. S. D. R. (2017). Quinoa: Nutritional, functional, and antinutritional aspects. *Critical Reviews in Food Science and Nutrition*, *57*(8), 1618-1630.

Fleet, G. H., & Zhao, J. (2018). Unravelling the contribution of lactic acid bacteria and acetic acid bacteria to cocoa fermentation using inoculated organisms. *International Journal of Food Microbiology*, *279*, 43-56.

Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., & Stokes, A. (2021). Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytologist*, *232*(3), 1123-1158.

Fuhrman, J. (2018). The hidden dangers of fast and processed food. *American Journal of Lifestyle Medicine*, *12*(5), 375-381.

Fukushima, A. R., Nicoletti, M. A., Rodrigues, A. J., Pressutti, C., Almeida, J., Brandão, T., & Souza Spinosa, H. D. (2016). Cassava Flour: Quantification of Cyanide Content. *Food and Nutrition Sciences*, *7*(7), 592–599.

Galili, G. (2002). New insights into the regulation and functional significance of lysine metabolism in plants. *Annual Review of Plant Biology*, *53*, 27-38.

Garcia-Yu, I. A., Garcia-Ortiz, L., Gómez-Marcos, M. A., Alonso-Dominguez, R., Gonzalez-Sanchez, J., Mora-Simon, S., & Recio-Rodriguez, J. I. (2018). Vascular and cognitive effects of cocoa-rich chocolate in postmenopausal women: a study protocol for a randomised clinical trial. *British Medical Journal Open*, *8*(12), e024095.

Gaudichon, C., & Calvez, J. (2021). Determinants of amino acid bioavailability from ingested protein in relation to gut health. *Current Opinion in Clinical Nutrition and Metabolic Care*, *24*(1), 55-58.

Ghribi, A. M., Gafsi, I. M., Blecker, C., Danthine, S., Attia, H., & Besbes, S. (2015). Effect of drying methods on physico-chemical and functional properties of chickpea protein concentrates. *Journal of Food Engineering*, *165*, 179-188.

Gibson, R. S., Raboy, V., & King, J. C. (2018). Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutrition Reviews*, *76*(11), 793-804.

Godswill, A. C. (2019). Proximate composition and functional properties of different grain flour composites for industrial applications. *International Journal of Food Sciences*, *2*(1), 43-64.

Gopinath, V., Saravanan, S., Al-Maleki, A. R., Ramesh, M., & Vadivelu, J. (2018). A review of natural polysaccharides for drug delivery applications: special focus on cellulose, starch and glycogen. *Biomedicine and Pharmacotherapy*, *107*, 96-108.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, *94*(3), 651-715.

Gulhane, P. R., & Jadhao, K. D. (2019). Evaluation of Cyanogenic Glycosides from some Medicinal Plants by Spectrophotometric Method. *Journal of Global Biosciences*, *8*(1), 5789-5794.

Gulukun, E. Z., Ari, M. M., & Alu, S. E. (2020). Proximate composition and antinutritional factors of differently processed kidney bean (*Phaseolus vulgaris*) seeds. *Nigerian Journal of Animal Production*, *47*(5), 169-175.

Gunathilake, K. G. T., Wansapala, M. A. J., & Herath, H. M. T. (2016). Comparison of nutritional and functional properties of mung bean (*Vigna radiate*) and cowpea (*Vigna unguiculata*) protein isolates processed by isoelectric precipitation. *International Journal of Innovative Research in Technology*, *3*(2), 139–148.

Guven, H., Arici, A., & Simsek, O. (2019). Flavonoids in our foods: a short review. *Journal of Basic and Clinical Health Sciences*, *3*(2), 96-106.

Gwana, A. M., Bako, M. M., Bagudu, B. Y., Sadiq, A. B., & Abdullahi, M. M. (2014). Determinations of phytochemical, vitamin, mineral and proximate compositions of varieties of watermelon seeds cultivated in Borno State, North-Eastern Nigeria. *International Journal of Nutrition and Food Sciences*, *3*(4), 238-245.

Hahn, S. K., Howland, A. K., & Terry, E. R. (1974). Cassava breeding at IITA. In: Colin Louis Avern Leakey Edition. *Proceedings of the International Tropical Root Crop Symposium. Ibadan, Nigeria, 2-9 December, 1973*, 4-10.

Hargreaves, M., & Spriet, L. L. (2020). Skeletal muscle energy metabolism during exercise. *Nature Metabolism*, *2*(9), 817-828.

Hasanuzzaman, A. T. M., Islam, M. N., Zhang, Y., Zhang, C. Y., & Liu, T. X. (2016). Leaf morphological characters can be a factor for intra-varietal preference of whitefly Bemisia tabaci (Hemiptera: Aleyrodidae) among eggplant varieties. *PLoS One*, *11*(4), e0153880.

Hennessy, R. C., Nielsen, S. D., Greve-Poulsen, M., Larsen, L. B., Sørensen, O. B., & Stougaard, P. (2020). Discovery of a bacterial gene cluster for deglycosylation of toxic potato steroidal glycoalkaloids α-chaconine and α-solanine. *Journal of Agricultural and Food Chemistry*, *68*(5), 1390-1396.

Hill, N. S., & Roberts, C. A. (2020). Plant Chemistry and Antiquality Components in Forage. *Forages: The Science of Grassland Agriculture*, *2*, 633-658.

Hirvonen, J., Liljavirta, J., Saarinen, M. T., Lehtinen, M. J., Ahonen, I., & Nurminen, P. (2019). Effect of phytase on *in vitro* hydrolysis of phytate and the formation of myo-inositol phosphate esters in various feed materials. *Journal of Agricultural and Food Chemistry*, *67*(41), 11396-11402.

Hohn, E., & Nell, A. (2017). Applying community health principles to veterinary practice. *Veterinary Record*, *180*(3), 73-75.

Howeler, R., Lutaladio, N., & Thomson, G. (2013). *Save and Grow Cassava A guide to Sustainable Production and intensification* (pp. 1-129). Rome, Italy: Food and Agriculture Organization of The United Nations.

Hwang, K. T., Kim, J. E., Park, J. N., & Yang, J. S. (2007). Effects of roasting, powdering and storing irradiated soybeans on hydrocarbon detection for identifying postirradiation of soybeans. *Food Chemistry*, *102*(1), 263-269.

Ibeabuchi, J. C., Okafor, D. C., Ahaotu, N. N., Eluchie, C. N., Agunwah, I. M., Chukwu, M. N., & Amandikwa, C. (2019). Effect of Dehulling on Proximate Composition and Functional Properties of Lima Bean (*Phaseolus lunatus*) Grown in Enugu State. *Journal of Food Research*, *8*(2), 116-121.

Ibegbulem, C. O., Igwe, C. U., Okwu, G. N., Ujowundu, C. O., Onyeike, E. N., & Ayalogu, E. O. (2013). Total amino acid profiles of heat-processed fresh *Elaeis guineensis* and *Raphia hookeri* wines. *Food Chemistry*, *138*(2-3), 1616-1620.

Imakumbili, M. L., Semu, E., Semoka, J. M., Abass, A., & Mkamilo, G. (2019). Soil nutrient adequacy for optimal cassava growth, implications on cyanogenic glucoside production: A case of konzo-affected Mtwara region, Tanzania. *PLoS One*, *14*(5), e0216708.

Indrastuti, Y. E., Estiasih, T., Christanti, R. A., Pulungan, M. H., & Zubaedah, E. (2018). Microbial and some chemical constituent changes of high cyanide cassava during simultant spontaneous submerged and solid-state fermentation of" gadungan pohung". *International Food Research Journal*, *25*(2), 487-498.

Inoue, M., Takeuchi, A., Horigane, S. I., Ohkura, M., Gengyo-Ando, K., Fujii, H., & Bito, H. (2015). Rational design of a high-affinity, fast, red calcium indicator RCaMP2. *Nature Methods*, *12*(1), 64-70.

Inyang, U. E., Akpan, E. O. & Bello, F. A., (2015) Effect of boiling and roasting on the nutrient and anti-nutrient contents in conophor nut flour. *International Journal of Information Research and Review*, *2*(6), 769-772.

Iragaba, P., Nuwamanya, E., Wembabazi, E., Baguma, Y., Dufour, D., Earle, E. D., & Kawuki, R. S. (2019). Estimates for heritability and consumer-validation of a penetrometer method for phenotyping softness of cooked cassava roots. *African Crop Science Journal*, *27*(2), 147-163.

Iriarte, J., Elliott, S., Maezumi, S. Y., Alves, D., Gonda, R., Robinson, M., & Handley, J. (2020). The origins of Amazonian landscapes: Plant cultivation, domestication and the spread of food production in tropical South America. *Quaternary Science Reviews, 248*, 106582-106596.

Ismaila, A. R., Alakali, J. S., & Atume, T. G. (2018). Effect of local processing techniques on the nutrients and anti-nutrients content of bitter cassava (Manihot Esculenta Crantz). *American Journal of Food Science and Technology*, *6*(3), 92-97.

Jaber, L. R., & Enkerli, J. (2016). Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. *Biological Control*, *103*, 187-195.

Jain, A., Subramanian, R., Manohar, B., & Radha, C. (2019). Preparation, characterization and functional properties of *Moringa oleifera* seed protein isolate. *Journal of Food Science and Technology*, *56*(4), 2093-2104.

James, S., Anuonye, J. C., Husseini, M., Ede, E. B., James, S. A., & James, Y. (2016). Chemical composition and functional properties of protein concentrate from selected cowpea seeds in Nigeria. *EC Nutrition*, *4*(3), 857-868.

Jarzebski, M. P., Ahmed, A., Boafo, Y. A., Balde, B. S., Chinangwa, L., Saito, O., & Gasparatos, A. (2020). Food security impacts of industrial crop production in subSaharan Africa: a systematic review of the impact mechanisms. *Food Security*, *12*, 105-135.

Jeelani, R., Maitra, D., Chatzicharalampous, C., Najeemuddin, S., Morris, R. T., & Abu-Soud, H. M. (2018). Melatonin prevents hypochlorous acid-mediated cyanocobalamin destruction and cyanogen chloride generation. *Journal of Pineal Research*, *64*(3), e12463.

Ježek, F., Kameník, J., Macharáčková, B., Bogdanovičová, K., & Bednář, J. (2020). Cooking of meat: effect on texture, cooking loss and microbiological quality–a review. *Acta Veterinaria Brno*, *88*(4), 487-496.

Jia, W., Rodriguez-Alonso, E., Bianeis, M., Keppler, J. K., & van der Goot, A. J. (2021). Assessing functional properties of rapeseed protein concentrate versus isolate for food applications. *Innovative Food Science and Emerging Technologies*, *68*, 102636-102832.

Jiang, H., Yu, F., Qin, L. I., Zhang, N., Cao, Q., Schwab, W., & Song, C. (2019). Dynamic change in amino acids, catechins, alkaloids, and gallic acid in six types of tea processed from the same batch of fresh tea (*Camellia sinensis L.*) leaves. *Journal of Food Composition and Analysis*, 77, 28-38.

Jo, H., Lee, J. Y., Cho, H., Choi, H. J., Son, C. K., Bae, J. S., & Lee, J. D. (2021). Genetic Diversity of Soybeans (*Glycine max* (L.) *Merr*.) with Black Seed Coats and Green Cotyledons in Korean Germplasm. *Agronomy*, *11*(3), 581-602.

John, K. M., Khan, F., Luthria, D. L., Garrett, W., & Natarajan, S. (2017). Proteomic analysis of anti-nutritional factors (ANF's) in soybean seeds as affected by environmental and genetic factors. *Food Chemistry*, *218*, 321-329.

Joshi, V. K. (2020). Value addition to Fruit and Vegetable Processing Waste–An Appraisal. *International Journal of Food and Fermentation Technology*, *10*(2), 35-58.

Joye, I. (2019). Protein digestibility of cereal products. *Foods*, *8*(6), 199-200.

Kadier, A., Ilyas, R. A., Huzaifah, M. R. M., Harihastuti, N., Sapuan, S. M., Harussani, M. M., & Ishak, M. R. (2021). Use of Industrial Wastes as Sustainable Nutrient Sources for Bacterial Cellulose (BC) Production: Mechanism, Advances, and Future Perspectives. *Polymers*, *13*(19), 3365-3476.

Kamuhu, R., Mugendi, B., Kimiywe, J., & Njagi, E. (2019). Proximate analysis of raw and roasted groundnut (*Arachis hypogaea* L.): Red Valencia and manikanta varieties. *International Journal of Food Science and Nutrition*, *4*(4), 191-194.

Kang, S. M., Kim, H. G., Yang, W. H., Yong, S. H., Park, D. J., Park, J. H., & Choi, M. S. (2017). Changes in the physicochemical characteristics of sancho oil according to the purification process. *Korean Journal of Medicinal Crop Science*, *25*(5), 296-304.

Kanwate, B. W., Ballari, R. V., & Kudre, T. G. (2019). Influence of spray-drying, freezedrying and vacuum-drying on physicochemical and functional properties of gelatin from *Labeo rohita* swim bladder. *International Journal of Biological Macromolecules*, *121*, 135-141.

Karami, H., Shariatifar, N., Nazmara, S., Moazzen, M., Mahmoodi, B., & Khaneghah, A. M. (2021). The Concentration and probabilistic health risk of potentially toxic elements (PTEs) in Edible Mushrooms (Wild and Cultivated) samples collected from different cities of Iran. *Biological Trace Element Research*, *199*(1), 389-400.

Karanja, P. N. (2017). Physicochemical Properties of Bamboo Shoots of Selected Species Grown in Kenya and Utilization as Human Food (Doctoral dissertation, Faculty of Agriculture, JKUAT).

Kariuki, S. M., Abubakar, A., Kombe, M., Kazungu, M., Odhiambo, R., Stein, A., & Newton, C. R. (2017). Burden, risk factors, and comorbidities of behavioural and emotional problems in Kenyan children: a population-based study. *The Lancet Psychiatry*, *4*(2), 136-145.

Khattab, R. Y., Arntfield, S. D., & Nyachoti, C. M. (2009). Nutritional quality of legume seeds as affected by some physical treatments, Part 1: Protein quality evaluation. *LWT-Food Science and Technology*, *42*(6), 1107-1112.

Kheddo, P., Tracka, M., Armer, J., Dearman, R. J., Uddin, S., Van Der Walle, C. F., & Golovanov, A. P. (2014). The effect of arginine glutamate on the stability of monoclonal antibodies in solution. *International Journal of Pharmaceutics*, *473*(1-2), 126-133.

Kinyua, M., & Okwaro, H. (2021). The Diversity of Induced M1V4 Mutant Population of Cassava (Mahinot Esculanta Crantz). *Journal of Advance Research in Food, Agriculture and Environmental Science*, *8*(4), 1-6.

Kirkensgaard, K. G. (2021). Saponins-amphipathic glycosides. *DNA, 2021*(29), 28-35.

Klotz, J. L. (2015). Bill E. Kunkle Interdisciplinary Beef Symposium: Physiologic effects of ergot alkaloids: What happens when excretion does not equal consumption? *Journal of Animal Science*, *93*(12), 5512-5521.

Kolapo, A. L., Salami, R. O., Onajobi, I., Oluwafemi, F., Fawole, A. O., & Adejumo, O. E. (2021). Detoxification and nutritional enrichment of cassava waste pulp using Rhizopus oligosporus and Aspergillus niger. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology*, *45*(1), 52-68.

Koubala, B. B., Laya, A., Massai, H., Kouninki, H., & Nukenine, E. N. (2015). Physicochemical characterization leaves from five genotypes of cassava (Manihot esculenta Crantz) consumed in the Far North Region (Cameroon). *American Journal of Food Science and Technology*, 3(2), 40-47.

Krisanapook, K., Anusornpornpong, P., & Luengwilai, K. (2019). Inflorescence and flower development in Thai aromatic coconut. *Journal of Applied Horticulture*, *21*, 3-12.

Krishnan, R., & Meera, M. S. (2018). Pearl millet minerals: effect of processing on bioaccessibility. *Journal of Food Science and Technology*, 55(9), 3362-3372.

Kulthe, A. A., Thorat, S. S., & Lande, S. B. (2016). Characterization of pearl millet cultivars for proximate composition, minerals and anti-nutritional contents. *Advances in Life Sciences*, 5(11), 4672-4675.

Kumar, V., Makkar, H. P. S., & Becker, K. (2011). Detoxified Jatropha curcas kernel meal as a dietary protein source: growth performance, nutrient utilization and digestive enzymes in common carp (*Cyprinus carpio* L.) fingerlings. *Aquaculture Nutrition*, *17*(3), 313-326.

Kumari, B., Hora, A., & Mallick, M. A. (2017). Stimulatory effect of PGPR (Plant Growth Promoting Rhizospheric Bacteria) on Medicinal and Growth properties of a potential medicinal herb *Chlorophytum borivilianum*: A review. *Journal of Plant Science and Research*, *33*(2), 151-156.

Kumari, N., Vinita, N. K., & Rani, P. (2018). Nutrient composition of full fat and defatted rice bran. *Asian Journal of Dairy and Food Research*, 37(1), 77-80.

Kunatsa, Y., Chidewe, C., & Zvidzai, C. J. (2020). Phytochemical and anti-nutrient composite from selected marginalized Zimbabwean edible insects and vegetables. *Journal of Agriculture and Food Research*, *2*, 100027-100037.

Kurzbaum, E., Iliasafov, L., Kolik, L., Starosvetsky, J., Bilanovic, D., Butnariu, M., & Armon, R. (2019). From the Titanic and other shipwrecks to biofilm prevention:

The interesting role of polyphenol-protein complexes in biofilm inhibition. *Science of The Total Environment, 658*, 1098-1105.

Kwakye, G. F., Jiménez, J., Jiménez, J. A., & Aschner, M. (2018). Atropa belladonna neurotoxicity: implications to neurological disorders. *Food and Chemical Toxicology*, *116*, 346-353.

Lamy, E., Rawel, H., Schweigert, F. J., Capela e Silva, F., Ferreira, A., Costa, A. R., & Sales-Baptista, E. (2011). The effect of tannins on Mediterranean ruminant ingestive behavior: The role of the oral cavity. *Molecules*, *16*(4), 2766-2784.

Latif, S., & Müller, J. (2015). Potential of cassava leaves in human nutrition: a review. *Trends in Food Science and Technology*, *44*(2), 147-158.

Lawal, S. O., Idowu, A. O., Malomo, S. A., Badejo, A. A., & Fagbemi, T. N. (2021). Effect of toasting on the chemical composition, functional and antioxidative properties of full fat and defatted sesame (*Sesamum indicum* L) seed flours. *Journal of Culinary Science and Technology*, *19*(1), 18-34.

Lawrence, A. (2021). Application of residual cyanide concentrations level in processed cassava (*Manihot esculenta*) to inhibit the growth of spoilage microorganisms in cooked rice (*Orysa sativa*). *International Journal of Phytology Research, 1*(2), 11-14.

Laya, A., Koubala, B.B., Kouninki, H. & Nukenine, E.N. (2018). Effect of harvest period on the proximate composition and functional and sensory properties of gari produced from local and improved Cassava (*Manihot esculenta*) varieties. *International Journal of Food Science*, *2018*, 1–15.

Le, H. T., Lie, K. K., Etayo, A., Rønnestad, I., & Sæle, Ø. (2021). Physical and nutrient stimuli differentially modulate gut motility patterns, gut transit rate, and transcriptome in an agastric fish, the ballan wrasse. *Plos One*, *16*(2), e0247076.

Lebot, V. (2019). *Tropical root and tuber crops: Cassava, sweet potato, yams, aroids* (pp. 1–413). Wallingford UK: Cabi Publishing.

Lebot, V., Champagne, A., Malapa, R., & Shiley, D. (2009). NIR determination of major constituents in tropical root and tuber crop flours. *Journal of Agricultural and Food Chemistry*, *57*(22), 10539-10547.

Lee, J. S., Chang, P. Y., Zhang, Y., Kizer, J. R., Best, L. G., & Howard, B. V. (2017). Triglyceride and HDL-C dyslipidemia and risks of coronary heart disease and ischemic stroke by glycemic dysregulation status: the strong heart study. *Diabetes Care*, *40*(4), 529-537.

Leinonen, I., Iannetta, P. P., Rees, R. M., Russell, W., Watson, C., & Barnes, A. P. (2019). Lysine supply is a critical factor in achieving sustainable global protein economy. *Frontiers in Sustainable Food Systems*, *3*, 27-30.

Leitão, G. G., Leal, C. M., Mendonça, S. C., & Pereda-Miranda, R. (2021). Purification of alkaloids by countercurrent chromatography. *Revista Brasileira de Farmacognosia*, *31*(5), 1-23.

Lemmens, L., Van Buggenhout, S., Van Loey, A. M., & Hendrickx, M. E. (2010). Particle size reduction leading to cell wall rupture is more important for the β-carotene bioaccessibility of raw compared to thermally processed carrots. *Journal of Agricultural and Food Chemistry*, *58*(24), 12769-12776.

Li, R., & Xiong, Y. L. (2021). Sensitivity of oat protein solubility to changing ionic strength and pH. *Journal of Food Science*, *86*(1), 78-85.

Li, S., Cui, Y., Zhou, Y., Luo, Z., Liu, J., & Zhao, M. (2017). The industrial applications of cassava: current status, opportunities and prospects. *Journal of the Science of Food and Agriculture*, *97*(8), 2282-2290.

Li, Y., Feng, X., Yu, X., Wang, Y., Liu, Y., Ye, X., & Chu, Q. (2020). Radix Tetrastigma flavonoids inhibit the migration and promote the apoptosis of A549 cells both in vitro and in vivo. *Journal of Functional Foods*, *72*, 104076-104178.

Lisanti, E., & Arwin, A. (2019). Phytochemical screening and proximate analysis of soybeans (*Glycine max*) variety Gamasugen 1 and Gamasugen 2 derived from gamma rays irradiation. *Journal of Physics Conference Series*, *1402*(5), 1-7.

Liu, K. L., Zheng, J. B., & Chen, F. S. (2017). Relationships between degree of milling and loss of Vitamin B, minerals, and change in amino acid composition of brown rice. *LWT-Food Science and Technology*, *82*, 429-436.

Liu, K., Zheng, J., Wang, X., & Chen, F. (2019). Effects of household cooking processes on mineral, vitamin B, and phytic acid contents and mineral bioaccessibility in rice. *Food Chemistry*, *280*, 59-64. Liu, H., Brettell, L. E., Qiu, Z., & Singh, B. K. (2020). Microbiome-mediated stress resistance in plants. *Trends in Plant Science*, *25*(8), 733-743.

Liu, Y., Yan, H., Zhang, X., Zhang, R., Li, M., Xu, T., & Zhao, J. (2020). Investigating the endophytic bacterial diversity and community structures in seeds of genetically related maize (*Zea mays* L.) genotypes. *3 Biotech*, *10*, 1-10.

Liu, X., Song, Q., Li, X., Chen, Y., Liu, C., Zhu, X., & Huang, J. (2021). Effects of different dietary polyphenols on conformational changes and functional properties of protein–polyphenol covalent complexes. *Food Chemistry*, *361*, 130071-130084.

Lu, H., Guo, L., Zhang, L., Xie, C., Li, W., Gu, B., & Li, K. (2020). Study on quality characteristics of cassava flour and cassava flour short biscuits. *Food Science and Nutrition*, *8*(1), 521-533.

Luo, Y., Shang, P., & Li, D. (2017). Luteolin: A flavonoid that has multiple cardioprotective effects and its molecular mechanisms. *Frontiers in Pharmacology*, *8*, 1-10.

Macfadyen, S., Tay, W. T., Hulthen, A. D., Paull, C., Kalyebi, A., Jacomb, F., & De Barro, P. (2021). Landscape factors and how they influence whitefly pests in cassava fields across East Africa. *Landscape Ecology*, *36*(1), 45-67.

Makinde, F.M., & Akinoso, R. (2014). Comparison between the nutritional quality of flour obtained from raw, roasted and fermented sesame (*sesamum indicum* l.) seed grown in Nigeria. *Acta Science Policy and Technology Alimentary*, *13*(3), 309319.

Malik, M. A., & Saini, C. S. (2018). Improvement of functional properties of sunflower protein isolates near isoelectric point: Application of heat treatment. *Learning with Technologies*, *98*, 411-417.

Mall, T. P. (2017). Diversity of underexploited pulses in Bahraich (Uttar Pradesh) India. *European Journal of Biomedical and Pharmaceutical Sciences*, *4*, 196-215.

Malla, N., Nørgaard, J. V., Lærke, H. N., Heckmann, L. H. L., & Roos, N. (2022). Some insect species are good-quality protein sources for children and adults: digestible indispensable amino acid score (DIAAS) determined in growing pigs. *The Journal of Nutrition*, *152*(4), 1042-1051.

Maphosa, Y., & Jideani, V. A. (2017). The role of legumes in human nutrition. M. Chavarri. *Functional Food Improve Health through Adequate Food*, *1*(3), 103–109.

Marczuk, J., Brodzki, P., Brodzki, A., & Kurek, Ł. (2018). The concentration of free amino acids in blood serum of dairy cows with primary ketosis. *Polish journal of veterinary sciences*, *21*(1), 149-156.

Martillanes, S., Rocha-Pimienta, J., Cabrera-Bañegil, M., Martín-Vertedor, D., & Delgado-Adámez, J. (2017). Application of phenolic compounds for food preservation: Food additive and active packaging. *Phenolic CompoundsBiological Activity*, *3*(8), 39-58.

Martínez-Pineda, M., Yagüe-Ruiz, C., Caverni-Muñoz, A., & Vercet-Tormo, A. (2019). Cooking legumes: a way for their inclusion in the renal patient diet. *Journal of Renal Nutrition*, *29*(2), 118-125.

Mayr, H. L., Thomas, C. J., Tierney, A. C., Kucianski, T., George, E. S., Ruiz-Canela, M., & Itsiopoulos, C. (2018). Randomization to 6-month Mediterranean diet compared with a low-fat diet leads to improvement in Dietary Inflammatory Index scores in patients with coronary heart disease: the AUSMED Heart Trial. *Nutrition Research*, *55*, 94-107.

McClements, D. J., & Grossmann, L. (2021). The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4), 4049-4100.

McMahon, J., Sayre, R., & Zidenga, T. (2022). Cyanogenesis in cassava and its molecular manipulation for crop improvement. *Journal of Experimental Botany*, *73*(7), 1853-1867.

Melesse, A., Masebo, M., & Abebe, A. (2018). The Substitution Effect of Noug Seed (Guizotia Abyssinica) Cake with Cassava Leaf (Manihot Escutulata C.) Meal on Feed Intake, Growth Performance, and Carcass Traits in Broiler Chickens. *Journal of Animal Husbandry and Dairy Sciences*, *2*, 1-9.

Melini, V., & Melini, F. (2021). Functional components and anti-nutritional factors in gluten-free grains: A focus on quinoa seeds. *Foods*, *10*(2), 351.

Mendonça, R. D., Carvalho, N. C., Martin-Moreno, J. M., Pimenta, A. M., Lopes, A. C. S., Gea, A., & Bes-Rastrollo, M. (2019). Total polyphenol intake, polyphenol subtypes and incidence of cardiovascular disease: The SUN cohort study. *Nutrition, Metabolism and Cardiovascular Diseases*, *29*(1), 69-78.

Mendoza-Avendaño, C., Meza-Gordillo, R., Ovando-Chacón, S. L., Luján-Hidalgo, M. C., Ruiz-Cabrera, M. A., Grajales-Lagunes, A., & Abud-Archila, M. (2019). Evaluation of bioactive and anti-nutritional compounds during soy milk fermentation with Lactobacillus plantarum BAL-03-ITTG and Lactobacillus fermentum BAL-21-ITTG. *Revista Mexicana de Ingeniería Química*, *18*(3), 967978.

Meuser, F., & Smolnik, H. D. (1980). Processing of cassava to gari and other foodstuffs. *Starch-Stärke*, *32*(4), 116-122.

Meybodi, N. M., Mirmoghtadaie, L., Sheidaei, Z., & Mortazavian, A. M. (2019). Wheat bread: potential approach to fortify its lysine content. *Current Nutrition and Food Science*, *15*(7), 630-637.

Meziani, S., Nadaud, I., Tahir, A., Nurit, E., Benguella, R., & Branlard, G. (2021). Wheat aleurone layer: a site enriched with nutrients and bioactive molecules with potential nutritional opportunities for breeding. *Journal of Cereal Science*, *100*, 103225.

Milke, L., Ferreira, P., Kallscheuer, N., Braga, A., Vogt, M., Kappelmann, J., & Marienhagen, J. (2019). Modulation of the central carbon metabolism of Corynebacterium glutamicum improves malonyl-CoA availability and increases plant polyphenol synthesis. *Biotechnology and Bioengineering*, *116*(6), 13801391.

Misra, N. N., Koubaa, M., Roohinejad, S., Juliano, P., Alpas, H., Inácio, R. S., & Barba, F. J. (2017). Landmarks in the historical development of twenty first century food processing technologies. *Food Research International*, *97*, 318-339.

Mohamed, E., Souhail, B., Olivier, R., Christophe, B., & Hamadi, A. (2007). Quality characteristics of sesame seeds and byproducts. *Food Chemistry*, *103*, 641-650.

Mohammed, A., Muhammad, I. U., Wudil, A. M., Alhassan, A. J., Abubakar, S. M., & Ngwen, A. L. (2019). Phytochemical screening and proximate analysis of root of *Curcuma longa Linn*. *European Journal of Pharmaceutical and Medical Research*, *6*, 138-141.

Moknatjou, R., Abbasian, S., Moghaddam, G., Shams-Ardekani, M. R., Toliyat, T., Sadeghpour, O., & Hajimahmoodi, M. (2015). Roasting effect on total cyanide, α-tocopherol and oil characteristics of the brown and yellow types of the flaxseed (*Linum usitatissimum* L.). *International Journal of Biosciences*, *6*(5), 273-282.

Moll, P., Grossmann, L., Kutzli, I., & Weiss, J. (2020). Influence of energy density and viscosity on foam stability–A study with pea protein (*Pisum Sativum* L.). *Journal of Dispersion Science and Technology*, *41*(12), 1789-1796.

Mombo, S., Dumat, C., Shahid, M., & Schreck, E. (2017). A socio-scientific analysis of the environmental and health benefits as well as potential risks of cassava production and consumption. *Environmental Science and Pollution Research*, *24*, 5207-5221.

Montagnac, J. A., Davis, C. R., & Tanumihardjo, S. A. (2009). Nutritional value of cassava for use as a staple food and recent advances for improvement. *Comprehensive Reviews in Food Science and Food Safety*, *8*(3), 181-194.

Morgan, N. K., & Choct, M. (2016). Cassava: Nutrient composition and nutritive value in poultry diets. *Animal Nutrition*, *2*(4), 253-261.

Mosayyebi, B., Imani, M., Mohammadi, L., Akbarzadeh, A., Zarghami, N., Edalati, M., & Rahmati, M. (2020). An update on the toxicity of cyanogenic glycosides bioactive compounds: Possible clinical application in targeted cancer therapy. *Materials Chemistry and Physics*, *246*, 122841-1421521.

Mosobalaje, M. A., Tewe, O. O., Adedoyin, A. A., Adebayo, B. F., & Oyegunwa, A. S. (2019). Some serum metabolites and haematological parameters of pullet chicks fed cassava root products (CRPs). *Journal of Agricultural Science and Technology A*, *9*, 151-156.

Mueller, B. S. D. F., Sakamoto, T., Silveira, R. D. D., Zambussi-Carvalho, P. F., Pereira, M., Pappas, G. J., & Vianello-Brondani, R. P. (2014). Differentially expressed genes during flowering and grain filling in common bean (*Phaseolus vulgaris*) grown under drought stress conditions. *Plant Molecular Biology Reporter*, *32*, 438-451.

Mühlen, G. S., Alves-Pereira, A., Carvalho, C. R. L., Junqueira, A. B., Clement, C. R., & Valle, T. L. (2019). Genetic diversity and population structure show different patterns of diffusion for bitter and sweet manioc in Brazil. *Genetic Resources and Crop Evolution*, *66*(8), 1773-1790.

Muleta, F., & Mohammed, A. (2016). Selecting and Introducing Preferable Methods of Cyanide Removal from Cassava Root in Selected Iodine Deficiency Disordered (IDD) Areas of Wolaita Zone. *Journal of Natural Sciences Research*, *6*(17), 22243186.

Mune, M. A. M., Minka, S. R., & Mbome, I. L. (2014). Optimising functional properties during preparation of cowpea protein concentrate. *Food chemistry*, *154*, 32-37.

Mushumbusi, C. B., Max, R. A., Bakari, G. G., Mushi, J. R., & Balthazary, S. T. (2020). Cyanide in cassava varieties and people's perception on cyanide poisoning in selected regions of Tanzania. *Journal of Agricultural Studies*, *8*(1), 180.

Mutungi, C., Irungu, F. G., Nduko, J., Mutua, F., Affognon, H., Nakimbugwe, D., & Fiaboe, K. K. M. (2019). Postharvest processes of edible insects in Africa: A review of processing methods, and the implications for nutrition, safety and new products development. *Critical Reviews in Food Science and Nutrition*, *59*(2), 276-298.

Nadjiam, D., Ayessou, N. C., & Guissé, A. (2020). Physicochemical Characterization of Nine Cassava (*Manihot esculenta Crantz*) Cultivars from Chad. *Food and Nutrition Sciences*, *11*(7), 741-756.

Nakabonge, G., Samukoya, C., & Baguma, Y. (2018). Local varieties of cassava: Conservation, cultivation and use in Uganda. *Environment, Development and Sustainability*, *20*(6), 2427-2445.

Nanbol, K. K., & Namo, O. (2019). The contribution of root and tuber crops to food security: A review. *Journal of Agricultural Science and Technology B*, *9*(4), 221233.

Nartey, F., & MØller, B. L. (1976). Amino acid profiles of cassava seeds (Manihot esculenta). *Economic Botany*, *30*(4), 419–423.

Nartey, F., Møller, B. L., & Andersen, M. R. (1973) The major constituents of *Manihot esculenta* Crantz seeds. *Tropical Science*, *15*, 273–273.

Nartey, F., Møller, B. L., & Andersen, M. R. (1974) Changes in the major constituents of *Manihot esculenta* Crantz seeds during germination and growth. *Economic Botany*, *28*, 145–145.

Nassar, N. (2000). Cytogenetics and evolution of cassava (*Manihot esculenta Crantz*). *Genetics and Molecular Biology*, *23*, 1003-1014.

Nassar, N. M., & Dorea, J. G. (1982). Protein content of cassava cultivars and its hybrid with wild Manihot species. *Turrialba*, *32*(4), 429-432.

Nathan, C., & Udosen, U. J. (2017). Comparative analysis of type 1 and type 2 cassava peeling machines. *Nigerian Journal of Technology*, *36*(2), 469-476.

Ndam, Y. N., Mounjouenpou, P., Kansci, G., Kenfack, M. J., Meguia, M. P. F., Eyenga, N. S. N. N., & Nyegue, A. (2019). Influence of cultivars and processing methods on the cyanide contents of cassava (Manihot esculenta Crantz) and its traditional food products. *Scientific African*, *5*, e00119.

Ndidi, U. S., Ndidi, C. U., Olagunju, A., Muhammad, A., Billy, F. G., & Okpe, O. (2014). Proximate, Antinutrients and Mineral Composition of Raw and Processed (Boiled and Roasted) Sphenostylis stenocarpa Seeds from Southern Kaduna, Northwest Nigeria. *International Scholarly Research Notices Nutrition*, *2014*, 1–9.

Ndubuisi, N., & Chidiebere, A. (2018). Cyanide in cassava a review. *International Journal of Genomics and Data Mining*, *2*(1), 1-10.

Nduwumuremyi, A., Melis, R., Shanahan, P., & Asiimwe, T. (2016). Participatory appraisal of preferred traits, production constraints and postharvest challenges for cassava farmers in Rwanda. *Food Security*, *8*(2), 375-388.

Neji, C., Semwal, J., Kamani, M. H., Máthé, E., & Sipos, P. (2022). Legume protein extracts: The relevance of physical processing in the context of structural, technofunctional and nutritional aspects of food development. *Processes*, *10*(12), 25862668.

Netto, A. B., Netto, C. M., Mahadevan, A., Taly, A. B., & Agadi, J. (2016). Tropical ataxic neuropathy–A century old enigma. *Neurology India*, *64*(6), 1151-2100.

Neunert, G., Górnaś, P., Dwiecki, K., Siger, A., & Polewski, K. (2015). Synergistic and antagonistic effects between alpha-tocopherol and phenolic acids in liposome system: spectroscopic study. *European Food Research and Technology*, *241*(6), 749-757.

Nguyen, N. T. T., Le, H. A. V., Pham, D. A., & Tran, T. N. Y. (2018). Evaluation of physical, nutritional and sensorial properties cookie supplied with Hibiscus sabdariffa L. seed powder (without shell). *International Food Research Journal*, *25*(3), 1281-1287.

Nielsen, R., & Huelsenbeck, J. P. (2001). Detecting positively selected amino acid sites using posterior predictive P-values. In *Biocomputing 2002*, 576-588.

Nigris, S., D'Apice, G., Moschin, S., Ciarle, R., & Baldan, B. (2021). Fleshy structures associated with ovule protection and seed dispersal in gymnosperms: a systematic and evolutionary overview. *Critical Reviews in Plant Sciences*, *40*(4), 285-302.

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., & Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. *Food Control*, *79*, 62-73.

Nissar, J., Ahad, T., Naik, H. R., & Hussain, S. Z. (2017). A review phytic acid: As antinutrient or nutraceutical. *Journal of Pharmacognosy and Phytochemistry*, *6*(6), 1554-1560.

Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science and Nutrition*, *6*(8), 2446–2458.

Nnamani, N. D., & Okonkwo, T. J. (2017). Optimization of metronidazole tablet formulation using Manihot utilissima starch and a combination of processing techniques. *Future Journal of Pharmaceutical Sciences*, *3*(1), 65-70.

Nunes, A. R., Vieira, Í. G., Queiroz, D. B., Leal, A. L. A. B., Maia Morais, S., Muniz, D. F., & Coutinho, H. D. M. (2018). Use of flavonoids and cinnamates, the main

photoprotectors with natural origin. *Advances in Pharmacological Sciences, 2018*, 1-9.

Nwafor, F. I., Egonu, S. N., Nweze, N. O., & Ohabuenyi, N. (2017). Effect of processing methods on the nutritional values and anti-nutritive factors of *Adenanthera pavonina* L. (Fabaceae) seeds. *African Journal of Biotechnology*, *16*(3), 106-112.

Nyirenda, K.K. (2020). Toxicity potential of cyanogenic glycosides in edible plants. *Medical Toxicology* (pp. 1–19). London, UK: IntechOpen Limited.

Ogbonna, A. C., Braatz de Andrade, L. R., Rabbi, I. Y., Mueller, L. A., Jorge de Oliveira, E., & Bauchet, G. J. (2021). Large-scale genome-wide association study, using historical data, identifies conserved genetic architecture of cyanogenic glucoside content in cassava (*Manihot esculenta Crantz*) root. *The Plant Journal*, *105*(3), 754-770.

Oghbaei, M., & Prakash, J. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food and Agriculture*, *2*(1), 1136015-1233110.

Ogunwolu, S. O., Henshaw, F. O., Mock, H. P., Santros, A., & Awonorin, S. O. (2009). Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale* L.) nut. *Food Chemistry*, *115*(3), 852-858.

Ohashi, A. Y. P., Pires, R. C. de M., Silva, A. L. B. de O., Santos, L. N. S. & Matsura, E. E. (2019). Minirhizotron as an In-Situ tool for assessing sugarcane root system growth and distribution. *Agricultural Research and Technology: Open Access Journal*, *21*(5), 1–4.

Ohimain, E. I. (2014). The prospects and challenges of cassava inclusion in wheat bread policy in Nigeria. *International Journal of Science, Technology and Society*, *2*(1), 6-17.

Ojo, M. A., Ade-Omowaye, B. I. O., & Ngoddy, P. O. (2018). Processing effects of soaking and hydrothermal methods on the components and in vitro protein digestibility of Canavalia ensiformis. *International Food Research Journal*, *25*(2), 720-729.

Oladeji, A. E., Pheneas, N., Prisca, C., Bukola, O., Ivor, M., & Busie, M. D. (2019). Nutritional quality of fritters produced from fresh cassava roots, high-quality cassava and soy flour blends, and consumer preferences. *Cogent Food and Agriculture*, *5*(1), 1677129-1677132.

Olagunju, A. I., & Ifesan, B. O. (2021). Changes in nutrient and antinutritional contents of sesame seeds during fermentation. *Journal of Microbiology, Biotechnology and Food Sciences, 2021*, 2407-2410.

Olagunju, O. F., Ezekiel, O. O., Ogunshe, A. O., Oyeyinka, S. A., & Ijabadeniyi, O. A. (2018). Effects of fermentation on proximate composition, mineral profile and

antinutrients of tamarind (*Tamarindus indica* L.) seed in the production of daddawa-type condiment. *LWT-Food Science and Technology*, *90*, 455-459.

Olaleye, A. A., Adeyeye, E. I., & Adesina, A. J. (2013). Chemical composition of bambara groundnut (V. *subterranea* L. *Verdc*) seed parts. *Bangladesh Journal of Scientific and Industrial Research*, *48*(3), 167-178.

Olasunkanmi, G. S., Omolayo, F. T., & Olusegun, O. T. (2017). Fatty acid profile, physico-chemical and functional properties of oil and protein isolate simultaneously extracted from sesame (*sesamum indicum*) seed. *Annals of Food Science and Technology*, *18*(1), 1-10.

Olawoye, B. (2016). Effect of Processing Methods on the Chemical Composition, Functional and Anti-oxidant Properties of *Amaranthus virides* Seed (Doctoral dissertation, Department of Food Science and Technology, Faculty of Technology, Obafemi Awolowo University, Ile-Ife).

Oleszek, M., Oleszek, W. (2020). Saponins in Food. *Handbook of Dietary Phytochemicals* (pp. 1–40). Singapore: Springer.

Oliviero, T., & Fogliano, V. (2016). Food design strategies to increase vegetable intake: The case of vegetable enriched pasta. *Trends in Food Science and Technology*, *51*, 58-64.

Oloyede, O. O., James, S., Ocheme, O. B., Chinma, C. E., & Akpa, V. E. (2016). Effects of fermentation time on the functional and pasting properties of defatted *Moringa oleifera* seed flour. *Food Science and Nutrition*, *4*(1), 89-95.

OM, A., & Emelike, N. J. T. (2018). Effect of varietal difference on the proximate, functional and sensory properties of melon seeds. *Research Journal of Food Science and Nutrition*, *3*(5), 59-64.

Omoruyi, F. O., Stennett, D., Foster, S., & Dilworth, L. (2020). New frontiers for the use of IP6 and inositol combination in treating diabetes mellitus: A review. *Molecules*, *25*(7), 1720-1932.

Omueti, O., Otegbayo, B., Jaiyeola, O., & Afolabi, O. (2009). Functional properties of complementary diets developed from soybean (*Glycine Max*), groundnut (*Arachis Hypogea*) and crayfish (*Macrobrachium* Spp). *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *8*(8), 563–573.

Onojah, P. K., & Odin, E. M. (2015). Cyanogenic glycoside in food plants. *International Journal of Innovation in Science and Mathematics*, *3*, 2347-9051.

Onwuka, G. I. (2005). Food analysis and instrumentation. *Theory and Practice* (pp. 140-146). Surulere, Lagos, Nigeria: Naphthalic prints.

Onyango, O. S. (2019). Development of Protein and Mineral Enriched Cassava Rootmillet-cowpea Leaves Composite Flour from Selected Popular Cassava Varieties in Kilifi and Taita-taveta Counties, Kenya (Doctoral dissertation, University of Nairobi).

Osadolor, N., & Isese, M. O. O. (2023). Crop-based agroforestry systems in the buffers of protected areas: Implications for tree species conservation in Okomu National Park, Nigeria. *Journal of Research in Forestry, Wildlife and Environment*, *15*(2), 94-104.

Ospina, M. A., Pizarro, M., Tran, T., Ricci, J., Belalcazar, J., Luna, J. L., & Becerra Lopez-Lavalle, L. A. (2021). Cyanogenic, carotenoids and protein composition in leaves and roots across seven diverse population found in the world cassava germplasm collection at CIAT, Colombia. *International Journal of Food Science and Technology*, *56*(3), 1343-1353.

Otekunrin, O. A., & Sawicka, B. (2019). Cassava, a 21st century staple crop: How can Nigeria harness its enormous trade potentials. *Acta Scientific Agriculture*, *3*(8), 194-202.

Paauw, M., Koes, R., & Quattrocchio, F. M. (2019). Alteration of flavonoid pigmentation patterns during domestication of food crops. *Journal of Experimental Botany*, *70*(15), 3719-3735.

Pakfetrat, S., Amiri, S., Radi, M., Abedi, E., & Torri, L. (2019). Reduction of phytic acid, aflatoxins and other mycotoxins in wheat during germination. *Journal of the Science of Food and Agriculture*, *99*(10), 4695-4701.

Panahi, S., Moghaddam, M. K., & Moezzi, M. (2020). Assessment of milkweed floss as a natural hollow oleophilic fibrous sorbent for oil spill cleanup. *Journal of Environmental Management*, *268*, 110688-112532.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, e47.

Panghal, A., Munezero, C., Sharma, P., & Chhikara, N. (2019). Cassava toxicity, detoxification and its food applications: a review. *Toxin Reviews*. *40*(1), 1-6.

Parmar, A., Sturm, B., & Hensel, O. (2017). Crops that feed the world: Production and improvement of cassava for food, feed, and industrial uses. *Food Security*, *9*(5), 907-927.

Parmenter, B. H., Croft, K. D., Hodgson, J. M., Dalgaard, F., Bondonno, C. P., Lewis, J. R., & Bondonno, N. P. (2020). An overview and update on the epidemiology of flavonoid intake and cardiovascular disease risk. *Food and Function*, *11*(8), 67776806.

Patel, B., Patel, G., Shah, S., & Parmar, S. (2017). A Review: *Coix lacryma jobi* L. *Research Journal of Pharmacognosy and Phytochemistry*, *9*(4), 248-252.

Patil, P., Thakur, A., Sharma, A., & Flora, S. J. S. (2020). Natural products and their derivatives as multifunctional ligands against Alzheimer's disease. *Drug Development Research*, *81*(2), 165-183.

Patterson, C. A., Curran, J., & Der, T. (2017). Effect of processing on antinutrient compounds in pulses. *Cereal Chemistry*, *94*(1), 2-10.

Pei, Y., Ai, T., Deng, Z., Wu, D., Liang, H., McClements, D. J., & Li, B. (2019). Impact of plant extract on the gastrointestinal fate of nutraceutical-loaded nanoemulsions: phytic acid inhibits lipid digestion but enhances curcumin bioaccessibility. *Food and Function*, *10*(6), 3344-3355.

Pena, J., Villot, A., & Gerente, C. (2020). Pyrolysis chars and physically activated carbons prepared from buckwheat husks for catalytic purification of syngas. *Biomass and Bioenergy*, *132*, 105435-105438.

Pereira, M. P., Corrêa, F. F., Polo, M., de Castro, E. M., Cardoso, A. Á., & Pereira, F. J. (2016). Seed germination of *Schinus molle* L. (Anacardiaceae) as related to its anatomy and dormancy alleviation. *Seed Science Research*, *26*(4), 351-361.

Petroski, W., & Minich, D. M. (2020). Is There Such a Thing as "Anti-Nutrients"? A Narrative Review of Perceived Problematic Plant Compounds. *Nutrients*, *12*(10), 2929-2958.

Phiri, S., Schoustra, S. E., van den Heuvel, J., Smid, E. J., Shindano, J., & Linnemann, A. R. (2020). How processing methods affect the microbial community composition in a cereal-based fermented beverage. *LWT-Food Science and Technology*, *128*, 109451-109452.

Pineda, M., Yu, B., Tian, Y., Morante, N., Salazar, S., Hyde, P. T., & Ceballos, H. (2020). Effect of pruning young branches on fruit and seed set in cassava. *Frontiers in Plant Science*, *11*, 1107-1117.

Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A. I. (2021). Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry*, *209*, 112891-212698.

Polthanee, A., Taboonmuang, R., & Manaonok, J. (2016). Root yield and nutrient removal of four cassava cultivars planted in early rainy season of northeastern thailand: Crop experienced to drought at mid-growth stage. *Asian Journal of Crop Science*, *8*(1), 24-38.

Popoola, T. O. S., & Yangomodou, O. D. (2006). Extraction, properties and utilization potentials of cassava seed oil. *Biotechnology*, *5*(1), 38-41.

Prosekov, A., Babich, O., Kriger, O., Ivanova, S., Pavsky, V., Sukhikh, S., & Kashirskih, E. (2018). Functional properties of the enzyme-modified protein from oat bran. *Food Bioscience*, *24*, 46-49.

Punyu, V. R. (2015). Palaeoproductivity Reconstruction for the Northern Indian Ocean Utilizing Lipid Biomarkers (Doctoral dissertation, Goa University).

Quijada-Morin, N., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2015). Extractability of low molecular mass flavanols and flavonols from

red grape skins. Relationship to cell wall composition at different ripeness stages. *Journal of Agricultural and Food Chemistry, 63*(35), 7654-7662.

Quinn, A. A., Myrans, H., & Gleadow, R. M. (2022). Cyanide content of cassava food products available in Australia. *Foods, 11*(10), 1384-1432.

Raboy, V., Gibson, R. S., Bailey, K. B., & King, J. C. (2020). Comparison of four methods for phytate analysis in plant-based foods. *Journal of Food Composition and Analysis, 90*, 10348-12324.

Rafii, M., Elango, R., Ball, R. O., Pencharz, P. B., & Courtney-Martin, G. (2018). Metabolic availability of the limiting amino acids lysine and tryptophan in cooked white African cornmeal assessed in healthy young men using the indicator amino acid oxidation technique. *The Journal of Nutrition, 148*(6), 917-924.

Rahate, K. A., Madhumita, M., & Prabhakar, P. K. (2021). Nutritional composition, antinutritional factors, pretreatments-cum-processing impact and food formulation potential of faba bean (*Vicia faba* L.): A comprehensive review. *LWT-Food Science and Technology, 138*, 110796-201697.

Rahman, M. M., Jahan, F. I., & Mim, S. A. (2019). A brief phytochemical investigation and pharmacological uses of citrus seed–a review. *Pharmacology OnLine, 1*, 94103.

Raji, M. O., Abegunde, P. T., Adams, T. O., Fasasi, M. O., Salako, A. I., Oso, A. O., & Bamgbose, A. M. (2021). Evaluation of nutrients composition, anti-nutritional factors and amino acid profiles of sundried cassava starch extract pulp. *Nigerian Journal of Animal Science and Technology, 4*(1), 111-118.

Raji, S. N., Subhash, N., Ravi, V., Saravanan, R., Mohanan, C. N., MakeshKumar, T., & Nita, S. (2016). Detection and classification of mosaic virus disease in cassava plants by proximal sensing of photochemical reflectance index. *Journal of the Indian Society of Remote Sensing, 44*, 875-883.

Rakotoarivony, R., Molia, S., Rakotomalala, E., Ramy-Ratiarison, R., Jori, F., & Pedrono, M. (2022). Bushpig (Potamochoerus larvatus) hunting in rural areas of Madagascar and its health and socioeconomic implications. *Frontiers in Conservation Science, 3*, 732626-743627.

Reut, M. S., & Płachno, B. J. (2020). Unusual developmental morphology and anatomy of vegetative organs in *Utricularia dichotoma*—Leaf, shoot and root dynamics. *Protoplasma, 257*(2), 371-390.

Ricci, A., Parpinello, G. P., & Versari, A. (2018). The Nutraceutical Impact of Polyphenolic Composition in Commonly Consumed Green Tea, Green Coffee and Red Wine Beverages: A Review. *Recent Advancement in Food Science and Nutrition Research, 2018*, 12-27.

Rihan, H. Z., Kareem, F., El-Mahrouk, M. E., & Fuller, M. P. (2017). Artificial seeds (principle, aspects and applications). *Agronomy, 7*(4), 71-75.

Rivadeneyra-Domínguez, E., & Rodríguez-Landa, J. F. (2020). Preclinical and clinical research on the toxic and neurological effects of cassava (*Manihot esculenta Crantz*) consumption. *Metabolic Brain Disease*, *35*(1), 65-74.

Rock, C. L., Flatt, S. W., Barkai, H. S., Pakiz, B., & Heath, D. D. (2017). Walnut consumption in a weight reduction intervention: effects on body weight, biological measures, blood pressure and satiety. *Nutrition Journal*, *16*(1), 1-10.

Rodsamran, P., & Sothornvit, R. (2018). Physicochemical and functional properties of protein concentrate from by-product of coconut processing. *Food Chemistry*, *241*, 364-371.

Rogers, D. J., & Fleming, H. S. (1973). A Monograph of Manihot esculenta: With an Explanation of the Taximetrics Methods Used. *Economic Botany*, *27*(1), 1-113.

Roman, L., Gomez, M., Li, C., Hamaker, B. R., & Martinez, M. M. (2017). Biophysical features of cereal endosperm that decrease starch digestibility. *Carbohydrate Polymers*, *165*, 180-188.

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition*, *2*(1), 1-14.

Sánchez-Pérez, R., Jørgensen, K., Olsen, C. E., Dicenta, F., & Møller, B. L. (2008). Bitterness in almonds. *Plant Physiology*, *146*(3), 1040-1052.

Sango, C., Marufu, L., & Zimudzi, C. (2016). Phytochemical, anti-nutrients and toxicity evaluation of Cleome gynandra and Solanum nigrum: Common indigenous vegetables in Zimbabwe. *British Biotechnology Journal*, *13*(3), 1-11.

Saranraj, P., Behera, S. S., & Ray, R. C. (2019). Traditional foods from tropical root and tuber crops: innovations and challenges. *Innovations in Traditional Foods* (pp. 159-191). Duxford, UK: Elsevier Inc.

Sathe, S. K., Zaffran, V. D., Gupta, S., & Li, T. (2018). Protein solubilization. *Journal of the American Oil Chemists' Society*, *95*(8), 883-901.

Sauer, J. D. (2017). *Historical geography of crop plants: A Selected Roster* (pp. 1–309). Boca Raton, FL, USA: CRC Press.

Savage, G., & Klunklin, W. (2018). Oxalates are found in many different european and asian foods-effects of cooking and processing. *Journal of Food Research*, *7*(3), 76-81.

Scheers, N., Rossander-Hulthen, L., Torsdottir, I., & Sandberg, A. S. (2016). Increased iron bioavailability from lactic-fermented vegetables is likely an effect of promoting the formation of ferric iron (Fe^{3+}). *European Journal of Nutrition*, *55*(1), 373-382.

Schiek, B., González, C., Mwendia, S., & Prager, S. D. (2018). Got forages? Understanding potential returns on investment in Brachiaria spp. for dairy

producers in Eastern Africa. *Tropical Grasslands-Forrajes Tropicales*, *6*(3), 117133.

Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., & Schwerdtle, T. (2019). Evaluation of the health risks related to the presence of cyanogenic glycosides in foods other than raw apricot kernels. *EFSA Journal*, *17*(4), e05662.

Schwarcz, J. (2014). *Is That a Fact?: Frauds, Quacks, and the Real Science of Everyday Life* (pp. 204-207). Toronto: Ecw Press.

Scott, G. J. (2021). A review of root, tuber and banana crops in developing countries: past, present and future. *International Journal of Food Science and Technology*, *56*(3), 1093-1114.

Sen, G., Sarkar, N., Nath, M., & Maity, S. (2020). Bioactive components of tea. *Archive of Food Nutritional Science*, *4*, 1-9.

Senbagalakshmi, P., Muthukrishnan, S., Jebasingh, T., Senthil Kumar, T., & Rao, M. V. (2019). Squalene, Biosynthesis and its role in production of bioactive compounds, a Proper Scientific Challenge–A Review. *Journal of Emerging Technologies and Innovative Research*, *6*, 505-526.

Senica, M., Stampar, F., Veberic, R., & Mikulic-Petkovsek, M. (2016). Processed elderberry (*Sambucus nigra* L.) products: A beneficial or harmful food alternative? *LWT-Food Science and Technology*, *72*, 182-188.

Serna-Saldivar, S.O., & Espinosa-Ramirez, J. Grain structure and grain chemical composition. *Sorghum and Millets: Chemistry, Technology and Nutritional Attributes* (pp. 85–130). Amsterdam, The Netherlands: AACC International Press.

Seth, O. (2020). A Review on the performance of some cassava peeling machines developed. *North American Academic and Research Journal*, *3*(2), 97-162.

Shaba, E. Y., Ndamitso, M. M., Tsado, J. M., Etsunyakpa, M. B., Tsado, A. N., & Muhammed, S. S. (2015). Nutritional and anti-nutritional composition of date palm (*Phoenix dactylifera* L.) fruits sold in major markets of Minna Niger State, Nigeria. *African Journal of Pure and Applied Chemistry*, *9*(8), 167–174.

Shahidi, F., & Dissanayaka, C. S. (2023). Phenolic-protein interactions: insight from insilico analyses–a review. *Food Production, Processing and Nutrition*, *5*(1), 1-21.

Shao, Y. Y., Lin, K. H., & Kao, Y. J. (2016). Modification of foaming properties of commercial soy protein isolates and concentrates by heat treatments. *Journal of Food Quality*, *39*(6), 695-706.

Sharifi-Rad, J., Rodrigues, C. F., Stojanović-Radić, Z., Dimitrijević, M., Aleksić, A., Neffe-Skocińska, K., & Calina, D. (2020). Probiotics: Versatile bioactive components in promoting human health. *Medicina*, *56*(9), 433.

Shi, L., Arntfield, S. D., & Nickerson, M. (2018). Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. *Food Research International*, *107*, 660-668.

Shivaraj, S. M., Vats, S., Bhat, J. A., Dhakte, P., Goyal, V., Khatri, P., & Deshmukh, R. (2020). Nitric oxide and hydrogen sulfide crosstalk during heavy metal stress in plants. *Physiologia Plantarum*, *168*(2), 437-455.

Simon, M. F., Mendoza Flores, J. M., Liu, H. L., Martins, M. L. L., Drovetski, S. V., Przelomska, N. A., & Kistler, L. (2022). Phylogenomic analysis points to a South American origin of Manihot and illuminates the primary gene pool of cassava. *New Phytologist*, *233*(1), 534-545.

Singh, B., Kumar, A., & Malik, A. K. (2017a). Flavonoids biosynthesis in plants and its further analysis by capillary electrophoresis. *Electrophoresis*, *38*(6), 820-832.

Singh, B., Singh, J. P., Shevkani, K., Singh, N., & Kaur, A. (2017b). Bioactive constituents in pulses and their health benefits. *Journal of Food Science and Technology*, *54*(4), 858-870.

Singh, I. P., Ahmad, F., Gore, D. D., Tikoo, K., Bansal, A., Jachak, S. M., & Jena, G. (2019). Therapeutic potential of seabuckthorn: a patent review (20002018). *Expert Opinion on Therapeutic Patents*, *29*(9), 733-744.

Singh, T. P., & Sogi, D. S. (2018). Comparative study of structural and functional characterization of bran protein concentrates from superfine, fine and coarse rice cultivars. *International Journal of Biological Macromolecules*, *111*, 281-288.

Sinha, K., & Khare, V. (2017). Review on: Antinutritional factors in vegetable crops. *The Pharma Innovation Journal*, *6*(12), 353-358.

Sirwi, A., & Hussain, M. M. (2018). Lipid transfer proteins in the assembly of apoBcontaining lipoproteins. *Journal of Lipid Research*, *59*(7), 1094-1102.

Smeriglio, A., Barreca, D., Bellocco, E., & Trombetta, D. (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British Journal of Pharmacology*, *174*(11), 1244-1262.

Soares, S., García-Estévez, I., Ferrer-Galego, R., Brás, N. F., Brandão, E., Silva, M., & de Freitas, V. (2018). Study of human salivary proline-rich proteins interaction with food tannins. *Food Chemistry*, *243*, 175-185.

Sokoła-Wysoczańska, E., Wysoczański, T., Wagner, J., Czyż, K., Bodkowski, R., Lochyński, S., & Patkowska-Sokoła, B. (2018). Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders—a review. *Nutrients*, *10*(10), 1561-2516.

Soyingbe, O. S., Ntanzi, C., Makhafola, T. J., & Kappo, A. P. (2020). Ameliorative effect of exogenously applied oxalic acid on nickel (heavy metal) induced stress in Zea mays. *Pakistan Journal of Botany*, *52*(2), 413-418.

Sozer, N., Holopainen-Mantila, U., & Poutanen, K. (2017). Traditional and new food uses of pulses. *Cereal Chemistry*, *94*(1), 66-73.

Sozer, N., Melama, L., Silbir, S., Rizzello, C. G., Flander, L., & Poutanen, K. (2019). Lactic acid fermentation as a pre-treatment process for faba bean flour and its effect on textural, structural and nutritional properties of protein-enriched glutenfree faba bean breads. *Foods*, *8*(10), 431-530.

Spencer, D. S., & Ezedinma, C. (2017). Cassava cultivation in sub-Saharan Africa. *Achieving Sustainable Cultivation of Cassava*, Cambridge, UK, Burleigh Dodds Science Publishing Limited, *1*, 141–157.

Streck, N. A., Pinheiro, D. G., Junior Zanon, A., Gabriel, L. F., Rocha, T. S. M., Souza, A. T. D., & Silva, M. R. D. (2014). Effect of plant spacing on growth, development and yield of cassava in subtropical environment. *Bragantia*, *73*, 407415.

Su, W., Zuo, T., & Peterson, T. (2020). Ectopic expression of a maize gene is induced by Composite Insertions generated through Alternative Transposition. *Genetics*, *216*(4), 1039-1049.

Subramaniam, S., Rather, S. A., Danda, S., & Pandey, A. K. (2022). The systematic significance of pod morphological and anatomical variations in Indian *Crotalaria* L.(Leguminosae). *Flora*, *290*, 152059-210950.

Sulaiman, N. S., Hashim, R., Amini, M. H. M., Danish, M., & Sulaiman, O. (2018). Optimization of activated carbon preparation from cassava stem using response surface methodology on surface area and yield. *Journal of Cleaner Production*, *198*, 1422-1430.

Sunil, L., Appaiah, P., Kumar, P. P., & Krishna, A. G. (2015). Preparation of food supplements from oilseed cakes. *Journal of Food Science and Technology*, *52*(5), 2998-3005.

Suo, H., Tian, R., Li, J., Zhang, S., Cui, Y., Li, L., & Sun, B. (2019). Compositional characterization study on high-molecular-mass polymeric polyphenols in red wines by chemical degradation. *Food Research International*, *123*, 440-449.

Swarnakar, A. K., Mohapatra, M., & Das, S. K. (2022). A review on processes, mechanisms, and quality influencing parameters for puffing and popping of grains. *Journal of Food Processing and Preservation*, *46*(10), e16891.

Tan, Z., Xie, Z., Dai, L., Zhang, Y., Zhao, H., Tang, S., & Hong, D. (2022). Genome- and transcriptome-wide association studies reveal the genetic basis and the breeding history of seed glucosinolate content in Brassica napus. *Plant Biotechnology Journal*, *20*(1), 211–225.

Tanaka, M., Yasuoka, A., Shimizu, M., Saito, Y., Kumakura, K., Asakura, T., & Nagai, T. (2017). Transcriptomic responses of the liver and adipose tissues to altered

carbohydrate-fat ratio in diet: an isoenergetic study in young rats. *Genes and Nutrition*, *12*(1), 1-12.

Thakur, A., Sharma, V., & Thakur, A. (2019). An overview of anti-nutritional factors in food. *International Journal of Chemical Studies*, *7*(1), 2472-2479.

Thakur, N. S., & Kumar, P. (2017). Anti-nutritional factors, their adverse effects and need for adequate processing to reduce them in food. *AgricINTERNATIONAL*, *4*(1), 56-60.

Tiwari, A. K. (2020). *Advances in Seed Production and Management*. *Advances in Seed Production and Management* (pp. 1–626). Singapore: Springer.

Toedt, J., Koza, D., & Van Cleef-Toedt, K. (2005). *Chemical composition of everyday products* (pp. 48-49). London: Greenwood Publishing Group.

Torres, J., Rutherfurd, S. M., Muñoz, L. S., Peters, M., & Montoya, C. A. (2016). The impact of heating and soaking on the in vitro enzymatic hydrolysis of protein varies in different species of tropical legumes. *Food Chemistry*, *194*, 377–382.

Tran, Q. B., Phenrat, T., & Lohitnavy, M. (2020). Physiologically based pharmacokinetic modeling of hydrogen cyanide in humans following the oral administration of potassium cyanide and cyanogenic glycosides from food. *Human and Ecological Risk Assessment: An International Journal*, *26*(6), 1496-1511.

Twinomuhwezi, H., Awuchi, C. G., & Rachael, M. (2020). Comparative study of the proximate composition and functional properties of composite flours of amaranth, rice, millet, and soybean. *American Journal of Food Science and Nutrition*, *6*(1), 6-19.

Ubalua, A. O., Ewa, F., & Okeagu, O. D. (2016). Potentials and challenges of sustainable taro (Colocasia esculenta) production in Nigeria. *Journal of Applied Biology and Biotechnology*, *4*, 53-59.

Ubwa, S. T., Adoga, S. O., Tyohemba, R. L., & Shambe, T. (2015). Quantitative Estimation of Hydrogen Cyanide in Fresh and Cooked Tuber Parenchyma (Pulp) of Three Cultivars of Sweet Cassava Cultivars Grown in Some Parts of Benue State, Nigeria. *Food and Nutrition Sciences*, *6*(10), 836-840.

Uchechukwu-Agua, A. D., Caleb, O. J., & Opara, U. L. (2015). Postharvest handling and storage of fresh cassava root and products: a review. *Food and Bioprocess Technology*, *8*(4), 729-748.

Ulbrich, T. C., Friesen, M. L., Roley, S. S., Tiemann, L. K., & Evans, S. E. (2021). Intraspecific variability in root traits and edaphic conditions influence soil microbiomes across 12 switchgrass cultivars. *Phytobiomes Journal*, *5*(1), 108120.

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., & Jaremko, M. (2020). Important flavonoids and their role as a therapeutic agent. *Molecules*, *25*(22), 5243-5333.

Umar, A. H. (2019). Modulatory role of antioxidant vitamins (a and e) in cyanide induced liver and brain damage in adult male albino mice. *Journal of Occupational Safety and Environmental Health Volume*, *4*(2), 57-67.

Uzoukwu, A. E., Ubbaonu, C. N., Nwosu, J. N., Ogueke, C. C., & Chukwu, M. N. (2020). The Anti-Nutritional and Proximate Composition of Rain Tree (*Samanea saman*) Pod Samples. *Agriculture and Food Sciences Research*, *7*(1), 28–37.

Vagadia, B. H., Vanga, S. K., & Raghavan, V. (2017). Inactivation methods of soybean trypsin inhibitor–A review. *Trends in Food Science and Technology*, *64*, 115–125.

Vashishth, A., Ram, S., & Beniwal, V. (2017). Cereal phytases and their importance in improvement of micronutrients bioavailability. *3 Biotech*, *7*(1), 42-44.

Veer, S. J., Pawar, V. S., & Kambale, R. E. (2021). Antinutritional factors in foods. *The Pharma Innovation Journal*, *1*(10), 1-4.

Verma, R., Prasad, R., & Gupta, A. (2017). Functional properties and health benefits in flaxseed fiber and oil (*linum usitatissimum* L.). *International Journal of Home Science*, *3*(1), 368-369.

Vishwakarma, B., & David, A. (2021). Underlying Risk Determinants of Acute and Moderate Malnutrition in Children and its Preventive Management. *Pharmaceutical and Biosciences Journal*, *9*(4), 1-11.

Vogiatzaki, E., Baroux, C., Jung, J. Y., & Poirier, Y. (2017). PHO1 exports phosphate from the chalazal seed coat to the embryo in developing Arabidopsis seeds. *Current Biology*, *27*(19), 2893-2900.

Vongcharoen, K., Santanoo, S., Banterng, P., Jogloy, S., Vorasoot, N., & Theerakulpisut, P. (2018). Seasonal variation in photosynthesis performance of cassava at two different growth stages under irrigated and rain-fed conditions in a tropical savanna climate. *Photosynthetica*, *56*(4), 1398-1413.

Wang, L., Gillis-Smith, S., Peng, Y., Zhang, J., Chen, X., Salzman, C. D., & Zuker, C. S. (2018). The coding of valence and identity in the mammalian taste system. *Nature*, *558*(7708), 127-131.

Wani, S. A., & Kumar, P. (2016). Effect of extrusion on the nutritional, antioxidant and microstructural characteristics of nutritionally enriched snacks. *Journal of Food Processing and Preservation*, *40*(2), 166-173.

Widuri, L. I., Lakitan, B., Sakagami, J., Yabuta, S., Kartika, K., & Siaga, E. (2020). Shortterm drought exposure decelerated growth and photosynthetic activities in chili pepper (*Capsicum annuum* L.). *Annals of Agricultural Sciences*, *65*(2), 149-158.

Wimalasekera, R. (2019). Effect of light intensity on photosynthesis. *Photosynthesis, Productivity and Environmental Stress* (pp. 65-73). Hoboken, NJ, USA: Wiley.

Wojtunik-Kulesza, K., Oniszczuk, A., Oniszczuk, T., Combrzyński, M., Nowakowska, D., & Matwijczuk, A. (2020). Influence of in vitro digestion on composition, bioaccessibility and antioxidant activity of food polyphenols—A non-systematic review. *Nutrients*, *12*(5), 1401-2511.

Wossen, T., Abdoulaye, T., Alene, A., Haile, M. G., Feleke, S., Olanrewaju, A., & Manyong, V. (2017). Impacts of extension access and cooperative membership on technology adoption and household welfare. *Journal of Rural Studies*, *54*, 223233.

Yin, J., Li, Y., Han, H., Liu, Z., Zeng, X., Li, T., & Yin, Y. (2018). Long-term effects of lysine concentration on growth performance, intestinal microbiome, and metabolic profiles in a pig model. *Food and Function*, *9*(8), 4153-4163.

Yoshiara, L. Y., Mandarino, J. M. G., Carrão-Panizzi, M. C., Madeira, T. B., da Silva, J. B., de Camargo, A. C., & Ida, E. I. (2018). Germination changes the isoflavone profile and increases the antioxidant potential of soybean. *Journal of Food Bioactives*, *3*, 144-150.

Ze, N. N., Engama, M.-J. M., & Ngang, J. J. E. (2021). New retting method of cassava roots improve sensory attributes of Bobolo and Chikwangue in Central Africa: an approach through just about right (JAR) test. *Emirates Journal of Food and Agriculture*, *33*(6), 475–482.

Zhang, J., Qiu, X., Tan, Q., Xiao, Q., & Mei, S. (2020). A comparative metabolomics study of flavonoids in radish with different skin and flesh colors (*Raphanus sativus* L.). *Journal of Agricultural and Food Chemistry*, *68*(49), 14463-14470.

Zhang, L., Sun, P., Huettmann, F., & Liu, S. (2022). Where should China practice forestry in a warming world?. *Global Change Biology*, *28*(7), 2461-2475.

Zhou, Z., Ringø, E., Olsen, R. E., & Song, S. K. (2018). Dietary effects of soybean products on gut microbiota and immunity of aquatic animals: a review. *Aquaculture Nutrition*, *24*(1), 644-665.

Zhu, F. (2015). Composition, structure, physicochemical properties, and modifications of cassava starch. *Carbohydrate Polymers*, *122*, 456-480.

Zhu, S. M., Lin, S. L., Ramaswamy, H. S., Yu, Y., & Zhang, Q. T. (2017). Enhancement of functional properties of rice bran proteins by high pressure treatment and their correlation with surface hydrophobicity. *Food and Bioprocess Technology*, *10*(2), 317-327.

APPENDICES

APPENDIX A: Essential Amino Acid Composition of Cassava Seed Protein Concentrate

Amino Acid	Concentration (g/100g protein)
Histidine	2.21
Isoleucine	3.79
Leucine	6.57
Lysine	4.60
Methionine	1.32
Phenylalanine	3.46
Threonine	2.92
Tryptophan	0.83
Valine	4.08

APPENDIX

B: Non-essential Amino Acid Composition of Cassava Seed Protein Concentrate

Amino Acid	Concentration (g/100g protein)
Arginine	5.26
Alanine	5.38
Aspartic acid	9.40
Cystine	1.58
Glutamic acid	13.78
Proline	4.23
Glycine	3.67
Serine	3.92
Tyrosine	2.93

APPENDIX

C: Sulphur Containing Amino Acid Composition of Cassava Seed Protein Concentrate

Amino Acid	Concentration (g/100g protein)
Methionine	1.32
Cysteine	1.58

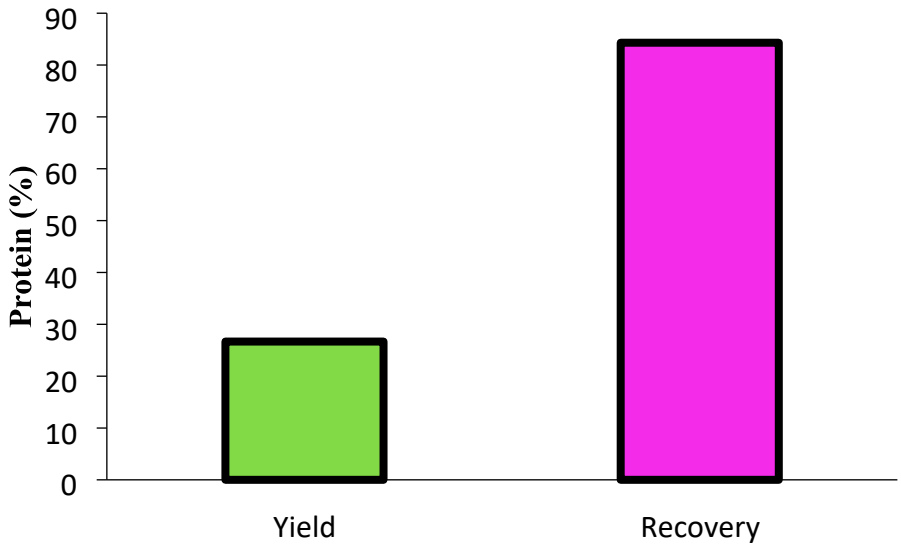
APPENDIX

D: Aromatic Amino Acid Composition of Cassava Seed Protein Concentrate

Amino Acid	Concentration (g/100g protein)
Tyrosine	2.93
Phenylalanine	3.46
Tryptophan	0.83

APPENDIX

E: Protein Yield and Recovery from Defatted Cassava Seed Flour.



APPENDIX

F: Cyanide Content of Cassava Seed Protein Concentrate using Different Processing Methods

Cyanide Content in Unprocessed Cassava Seed Protein Concentrate (mg/100g)	Processing Methods	Processing Time	Cyanide Reduction (mg/100g)	% Change in Cyanide Content
1008.43 ± 9.30	Fermentation	3 days	311.03	69.16
		4 days	269.51	73.27
		5 days	213.01	78.87
	Roasting	180^0c; 30mins	318.99	68.37
		180^0c; 40mins	212.03	78.97
		180^0c; 50mins	195.70	80.59
	Boiling	100^0c; 30mins	310.56	69.20
		100^0c; 1hr	144.29	85.69
		100^0c; 2hrs	81.35	91.93
		100^0c; 18hrs	39.18	96.11
		100^0c; 20hrs	16.90	98.32
		100^0c; 22hrs	8.61	99.15
		100^0c; 24hrs	2.98	99.70
		100^0c; 26hrs	0.91	99.90

APPENDIX

G: Unpeeled Raw Cassava Seeds

APPENDIX

H: Peeled Cassava Seeds

APPENDIX

I: Milled Cassava Seed Flour

APPENDIX

J: Cassava Seed Protein Concentrate