EVALUATION OF NUTRITIONAL, BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF UNPROCESSED AND PROCESSED FLOURS FROM THREE SELECTED UNDERUTILIZED SEEDS IN RATS

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ABSTRACT

In view of prevalent food shortages in most countries including Nigeria, ensuring food security has become an issue of key importance to countries with different degrees of economic development; hence attention is currently being drawn to the exploitation of underutilized and unconventional plant foods. The nutritional, biochemical and functional properties of flours from unprocessed and processed watermelon (*Citrullus lanatus*), moringa (*Moringa oleifera Lam.*) and fluted pumpkin (*Telfaira occidentalis Hook. F*) seeds were investigated. Flours were produced from soaked, germinated and boiled *Citrullus lanatus*, *Moringa oleifera Lam.* and *Telfaira occidentalis* seeds. Standard methods of analyses were used to determine their nutritional, functional, antinutritional and biochemical properties. Animal feed was compounded using each of the seed flours as a protein source in place of groundnut. The wistar rats were divided into thirteen (13) groups of six (6) per group and allocated to thirteen dietary treatments which are soaked watermelon seed flour (SWMSF), germinated watermelon seed flour (GWMSF), boiled watermelon seed flour (BWMSF), unprocessed watermelon seed flour (UWMSF), soaked moringa seed flour (SMSF), germinated moringa seed flour (GMSF), boiled moringa seed flour (BMSF), unprocessed moringa seed flour (UMSF), fluted pumpkin soaked fluted pumpkin seed flour (SFSF), germinated fluted pumpkin seed flour (GFSF), boiled fluted pumpkin seed flour (BFSF), unprocessed fluted pumpkin seed flour (UFSF) and groundnut cake served as control diet). After twenty eight (28) days of feeding, the rats were euthanized and blood samples were collected for biochemical and heamatological parameters while kidney and liver of rats were excised for histopathological analysis. The results revealed that soaking and boiling significantly ($p < 0.05$) increased the protein (16.90 % and 18.77 % respectively) and fat (29.05 % and 26.82 % respectively) contents of wetermelon seed flour. Germination significantly ($p < 0.05$) increased the protein content of moringa (32.13 %) and fluted pumpkin (25.70 %). Significant ($p < 0.05$) increase was observed in the carbohydrate contents of all the processed watermelon seed flours, significant ($p < 0.05$) increase was observed in soaked and boiled moringa seed flours while significant ($p <0.05$) decrease in carbohydrate contents was observed in all processed seed flours of fluted pumpkin. Fibre content was significantly ($p < 0.05$) decreased in all processed seed flours of watermelon, no significant ($p > 0.05$) difference was observed in all seed flours of moringa but significant ($p < 0.05$) increase was observed in fluted pumpkin seed flour when compared to their respective unprocessed seeds. Also, processing significantly (p

i. 0.05) decreased the antinutriet (tannins, saponins, cyanogenic glycoside, oxalate, phytate and trypsin inhibitor) composition of all the seed flours when compared to their respective unprocessed seed flours. Significant improvement in the functional properties of all the processed seed flours was observed. Significant ($p < 0.05$) elevation was also observed in some heamatological parameter of rats fed with supplemented diets of the processed seed flours while reduction was observed in all serum liver enzymes (AST, ALT and ALP) and bilirubin except for ALP of boiled moringa group where an elevation was seen. Serum creatinine levels of rats fed processed seed flours supplemented diet were significantly ($p < 0.05$) reduced, showing a better outcome interms of heamatological parameters and liver biomarkers. Histopathological examination of the liver of rats fed supplemented unprocessed seed fluted pumpkin revealed confluence fenthery degeneration of the hepatocyte but normal histoarchitecture of kidney and liver was observed in all other groups. This study revealed that processing of these underutilized seeds (watermelon, moringa and fluted pumpkin) had improved the nutrient quality, bioavailability and functional properties of the seeds recommending that they could be used in food formulations.

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ABBREVIATIONS, GLOSSARIES AND SYMBOLS

FAO	Food and Agricultural Organization
UNICEF	United Nation International Children Emergency Fund
LDL	Low-Density Lipoprotein
HDL	High-Density Lipoprotein
TMAO	Trimethylamine N-Oxide
ATP	Adenosine Triphosphate
PCV	Pack Cell Volume
MCHC	Mean Corpuscular Haemoglobin Concentration
ALT	Alanine Aminotransferase
PYOD	Pyruvate Oxidase
GPT	Glutamate Pyruvate Transferase
AST	Aspartate Aminotransferase
ALP	Alkaline Phosphatase
MCV	Mean Cell Volume
CCL_4	Carbon Tetrachloride
MCH	Mean Cell Hemoglobin
PT	Prothrombin Time
APTT	Activated Partial Thromboplastin Time
AOAC	Association of Official Analytical Chemist
EDTA	Ethylene Diamine Tetraacetate
WBC	White Blood Cell Count
RBC	Red Blood Cell Count
Hb	Haemoglobin Concentration
DPX	Dibutylphthalate Xylene

SWMSF	Soaked Watermelon Seed Flour
GWMSF	Germinated Watermelon Seed Flour
BWMSF	Boiled Watermelon Seed Flour
RWMSF	Unprocessed Watermelon Seed Flour
SMSF	Soaked Moringa Seed Flour
GMSF	Germinated Moringa Seed Flour
BMSF	Boiled Moringa Seed Flour
RMSF	Unprocessed Moringa Seed Flour
SFSF	Fluted Pumpkin Soaked Fluted Pumpkin Seed Flour
GFSF	Germinated Fluted Pumpkin Seed Flour
BFSF	Boiled Fluted Pumpkin Seed Flour
RFSF	Unprocessed Fluted Pumpkin Seed Flour
TI	Trypsin Inhibitor
CG	Cyanogenic Glycoside
WAC	Water Absorption Capacity
OAC	Oil Absorption Capacity
BD	Bulk Density
EC	Emulsion Capacity
FC	Foaming Capacity
FS	Foaming Stability
ES	Emulsion Stability
TB	Total Bilirubin,
CB	Conjugated Bilirubin
L	Lymphocytes
MONO	Monocytes
ECO	Eosinophils

NEU	Neutrophils
BASO	Basophils

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Good nutrition means getting all required nutrients in the right proportion. Nutrient could be preserved or improved through proper processing methods and efficient storage techniques; and these could also reduce the risk of food borne illness (Longland *et al.*, 2016). Protein, one of the important dietary components is seen as the most expensive component of any diet.

Proteins can be derived from both plant and animal sources. Aside the sources, the quality of protein contained in a diet is also important. Protein is required for growth and maintenance of tissue, biochemical reaction and serve as hormones, helps in regulations of concentration of acids and bases in the blood and other bodily fluids, transport nutrients through out the body and serve as antibodies to protect the body from foreign invaders (Gavin, 2018).

Therefore, deficiency of protein in diet could lead to various health problems such as kwashiorkor, fatty liver, stunted growth, loss of muscle mass, brittle nails and hair loss and so on (Gavin, 2018). In most developing countries protein deficiency is as a result of consumption of insufficient protein in comparism with requirement due to high cost of animal proteins and conventional food rich in protein (Mariotti and Gardner, 2019), neccessitate alternative source of proteins, which is why attention is the main reason for shifting to underutilize seeds.

Hunger and malnutrition are major problems in most developing countries including Nigeria. Increased population growth, poverty, fall in agricultural production and

ignorance have been identified as some of the causes of food shortage and the associated malnutrition. This results in severe consequences on human well-being by aggravating poverty, irreversible mental damages, poor growth among children, decreased future earnings of individuals and decreased growth of a country resulting in decrease in gross national product due to short life span (FAO, 2009). Nigeria is a very populous nation with over 213 million people in 2021 with a high fertility rate of about five children per woman (UNICEF, 2022), and their sound health can only be achieved by eating proper kinds of food.

Well-balanced human and animal diets should contain adequate amount of nutrients (Habib *et al*., 2015), lack or shortage of these nutrients (protein, carbohydrate, fat, vitamin and mineral) could lead to development of health problems such as certain types of cancer, rickets, stunted growth in children, cardiovascular diseases, malnutrition, type 2 diabetes, obesity and other nutrient related diseases (Aranceta, 2004). Reports indicated that protein deficiency is the commonest form of malnutrition in developing countries (Dipasquale *et al*., 2020).

In view of prevalent food shortages, highest priority should therefore be given to providing sufficient protein for its citizens in order to increase national food supplies. That is why; attention is currently being drawn to the exploitation of underutilized and unconventional plant resources. Some of the underutilized plant seeds may fit well into subsistence agriculture as alternative protein sources. There are many parts of plants available, which are rich in nutrients, one of which is plant seeds.

Seeds are embryonic stage of the plant life cycle enclosed in a protective outer covering. The formation of the seed is part of the process of reproduction in seed plants. They have nutritive and calorific values, which make them to be richly pack with healthy

plant fat, fibre, protein, vitamins, minerals and antioxidants (Kelly, 2015). Thus they play important roles as sources of lipids, proteins, carbohydrates and other nutrients in human diet, which are necessary for maintaining proper health (Adeleke and Babalola, 2020).

Previous research has shown that seeds contain nutritionally important bio- compounds and other phyto-compounds (Omorayi and Dilworth, 2007). They are the major and dominant source of human calories, proteins, mineral and trace element (such as iron, calcium, magnesium, potassium, zinc, copper, and selenium), vitamins (A, E and some B group) and fats (omega 3 and omega 6 fatty acids). These are required for the proper functioning of the heart, circulatory and immune system. They are good for intestinal health, prevention of colon cancer, gluten- free and in weight management (Riccardo, 2015). Examples of some of these seeds are sesame seeds, watermelon seeds, flax seeds, chia, hemp seed, fluted pumpkin seed, sunflower seed, moringa seeds, peanut, walnut and so on. The seeds considered in this research work are watermelon (*Citrullus lanatus*), moringa (*Moringa oleifera*) and fluted pumpkin (*Telfaira occidentalis*).

Manika *et al*. (2015) reported that Indian watermelon seed is highly rich in protein (39.12 %), carbohydrate, vitamins, mineral and fats especially polyunsaturated fatty acids, Taiwo *et al*. (2008) also reported that seed of watermelon is a good source of fiber which can helps maintain bowel regularity and works to prevent colon and renal cancer.

The nutritional composition of fluted pumpkin seed revealed that it contains protein (31 %), fat (53 %), fibre (3 %), carbohydrate (15 %), moisture 2 % and ash (2 %) (Kuku *et al*., 2014). Fluted pumpkin seed is known for its antioxidant, antiplasmodial, antimicrobial and antidiabetic activities (Kayode and Kayode, 2010).

Indiana moringa seeds is known for its high protein (about 39.12 %), vitamins, mineral, carbohydrate and high oil content (about 42 %), the coagulant of seeds could also be used for wastewater treatment (Foidl *et al*., 2001). Moringa seeds extract has been reported to be known for its anti-inflammatory, anti-spasmodic , anti- hypertensive, anti-tumor, anti- oxidant, anti-pyretic, anti-ulcer, anti-epileptic, diuretic, cholesterol lowering, anti-diabetic and hepatoprotective activities (Lai *et al*., 2010; Paliwal *et al*., 2011; Sharma *et al*., 2012; Huang *et al*., 2012b).

1.2 Statement of the Research Problem

Food insecurity, food shortage and undernutrition remain serious problems in many countries despite various measures taken to alleviate the world hunger problem (FAO, 2020). Poverty, war and conflict, natural disasters and climate change, as well as population growth are considered to be the main causes of hunger and malnutrition (FAO, 2020). According to the most recent Food and Agriculture Organization of the United Nations (FAO) data, around 13 % of the population living in developing countries are suffering from undernourishment (FAO, 2020), making feeding the world's population a challenge that is likely to become even more serious in the future (UNICEF 2021). Food production needs to double by 2050 to meet the increasing demand hence the need to explore nutrient potentials of underutilized seeds.

Seeds, being a rich source of essential nutrients and antioxidants, are the key component of the balanced human diet and also the main drivers in achieving global nutritional security by providing nutrients (Sati *et al.*, 2020). According to Reshmy *et al.* (2021) seeds that are often discarded while their fruits are eaten, may be termed as underutilized seeds. These underutilized seeds despite their high protein content are annually wasted only to be replanted; they are neglected due to lack of in-depth

knowledge about their performance and input requirements nutritional and industrial potentials.

Global population growth has continuously increase the demand for food; leading to food shortage and malnutrition, particularly in developing countries (FAO, 2011). The daily intake falls below minimum energy requirement leading to physical symptoms caused by energy and nutrient deficiencies resulting from an inadequate or unbalanced diet, or from inability of the body to use food effectively because of infection or disease. In developing countries protein energy malnutrition and micronutrient deficiencies have continued to be serious problems due to food shortage and high prices of food, particularly foods of animal origin (Jacob *et al*., 2013).

In view of prevalent food shortages and the need to increase national food supplies, it may be worthwhile to look at the biavailabilities of these seeds. Thus this study tends to explore the potentials of some underutilized seeds.

1.3 Justification of the Study

Many underutilized seeds are very rich sources of vitamins, minerals, high antioxidant activity, and other nutrients such as carbohydrates, proteins, and fats. They are usually cheap and readily available (Sati *et al.,* 2020). Thus, they could also be used as animal feed, oil source or for therapeutic purposes.

Focusing on proper processing methods of the underutilized seeds is an effective way to improve the bioavailability of the nutrients. With the use of underutilized seeds, there will be reduced risk of over-reliance on a very limited number of major crops and animal proteins. Besides, they can contribute to sustainable livelihoods through household food security as they can widen the food edibility options.

Watermelon (*Citrullus lanatus*) seeds are often discarded while the fruit is eaten. However, previous studies have shown that watermelon seeds are rich sources of proteins and fat, in addition to its theurapeutic value (Kausar *et al.,* 2020). One major challenge of the seed is that it has been reported to be rich in some antinutrient such as saponins, tannins, cyanogenic glycosides and oxalate were also present (Manika *et al*., 2015).

Moringa (*Moringa oleifera*) seeds have been reported for its medicinal uses and high nutritional value (Santos *et al*., 2005), which make them fit to combat malnutrition in infant and nursing mothers. Just like watermelon seed it is as rich in saponins, cyanogenic glycosides and oxalate (Alessandro *et al*., 2016).

Fluted pumpkin (*Telfairia occidentalis)* is widely consumed in Nigeria, despite its high protein content, it is annually wasted only to be replanted (Fagbemi, 2007). Kuku *et al*. (2014) reported that fluted pumpkin seeds are rich in essential nutrients but major limitation in the utilization of *T. occidentalis* seeds is that they contain anti-nutritional factors such as enzyme inhibitors, allergens, lectins, and other naturally occurring substances that may influence diet intake, digestibility, absorption and metabolic processes in animals and humans.

This study will therefore provide in-depth information on the effect of processing on the nutrient composition of underutilized seeds and study their inclusion in diets of experimental animals.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this study is to evaluate the nutritional, biochemical and functional properties of unprocessed and processed flours from three selected underutilized seeds in rats.

1.4.2 Objectives

The objectives are to determine the effects of processing methods on the:

ii. nutritional composition of flour from watermelon, moringa and fluted pumpkin seeds.

iii. antinutrient composition of flour from the seeds.

iv. functional properties of flour from the seeds.

v. weight changes of albino wistar rats fed diet supplemented with flour of the seeds

vi. biochemical and heamatological parameters in rats fed diet supplemented with flour of the seeds.

vii. histological architecture of liver and kidney of the rats fed diet supplemented with flour of the seeds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General Description of Seeds

A seed can be referred to as embryonic plant enclosed in a protective outer covering. The formation of the seed is part of the process of reproduction in seed plants, they are the product of the ripened ovule, after fertilization by pollen and some growth within the mother plant (Kelly, 2015). Some seeds are suitable for human or animal consumption because they are dominant source of human calories and protein, they also provide most cooking oils, many beverages and spices and some important food additives (Adeleke and Babalola, 2020).

Seeds often contain chemical compounds to discourage herbivores and predators. These compounds taste bad and are toxic or break down into toxic compounds within the digestive system and these substances influence diet intake, digestibility, absorption and metabolic processes in animals and humans (Adeleke and Babalola, 2020). Children, being smaller than adults, are more susceptible to poisoning by plants and seeds (Adeleke and Babalola, 2020).

2.1.1 Watermelon seeds

Watermelon, (*Citrullus lanatus*) is a fruit crop, an herbaceous creeping plant belonging to the family Cucurbitaceae. It is a vine-like flowering plant. It is a berry having a thick rind (exocarp) and fleshy center (mesocarp and endocarp), is mainly propagated by seeds, and thrives best in warm areas. It is a tropical plant and requires a lot of sunshine and high temperature of over 25 °C for optimum growth (Betty *et al*., 2016). It contains about 93 % water hence named watermelon (Baker *et al.,* 2012). The fruit is known to be a good source of lycopene and carotenoid. It helps quench the free radicals that

contribute to conditions like asthma, atherosclerosis, diabetes, colon cancer and arthritis. It is also high in fibre, citrulline and amino acid that the body uses to make arginine (Oyeleke *et al*., 2012).

Watermelon seeds are of different varieties vary in size, thickness, texture of the seeds coat and the thickness of the seeds edges. There are small, moderate and large sized seeds. The seeds differ in colour, they may be black, brown, red, yellow or rarely white (Milala *et al.,* 2018), watermelon seeds length and width may be about (0.6 – 1.5 cm) and (0.5 – 0.7 cm) respectively. Watermelon seeds are known to be highly nutritional; they are rich sources of protein, vitamins B, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others as well as phytochemicals (Braide *et al*., 2012). The seeds of watermelons are known to have economic benefits especially in countries where cultivation is on the increase. The seeds are milled into flour and used to prepare snacks and sauces. Oil derived from the seeds is used in cooking and in production of cosmetics (Jensen *et al*., 2011).

Betty *et al*. (2016) reported that watermelon seeds had moisture content of 7.40 % - 8.50 %; fat, 26.50 % - 27.83 %; protein, 16.33 % - 17.75 %; fibre, 39.09 % - 43.28 %; ash, 2.00 % - 3.00 %; carbohydrate, 9.55 % - 15.32 % and energy value of 354.05 - 369.11 kcal / 100 g. The seeds also contained appreciable minerals (calcium, phosphorus, magnesium, sodium, potassium and zinc) with potassium ranges between 3.40 mg/100 g to 3.5 mg/100 g being the highest while sodium (0.07 - 0.08 mg/100 g), saponins, tannins, triterpenoids glycosides and alkaloids were also present.

Manika *et al*. (2015) also reported that the crude protein content of a whole water melon seed is 34.22 %, 31.999 % fat content, 26.57 % carbohydrate content, high energy value of 531.151 Kcal/ 100 g, 0.1 % of dietary fiber, water melon seeds contain 3.575 % moisture and ash content of 3.636 %. The mineral analysis of water melon seeds reveals

that they are rich in calcium (444 ppm), potassium (6520 ppm), magnesium (3090 ppm) and phosphorus (6630 ppm) (Manika *et al.*, 2015).

2.1.2 Fluted pumpkin seeds

Fluted pumpkin (*Telfairia occidentalis*) is a perennial plant food with great economic importance in Nigeria, that belongs to the family of *Cucurbitaceae*, a creeping vegetative that spreads low across the ground with large lobed leaves, and long twisting tendrils (Ajayi *et al*., 2013). The harvesting of fluted pumpkin usually takes place 120-150 days after sowing. The popularity of the plant stems from the high nutritional value of its leaf and seed, which are eaten. Its leaves are rich in protein, oil, vitamins and minerals, folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A, C and K but low in crude fibre (Ladeji *et al.*, 1995). They are also rich in iron and have been reported to be useful in the treatment of anaemia (Alada, 2000). Chemically, *T. occidentalis* leaf extract contains 21.31 % crude protein, 6.41 % crude fibre, 5.50 ether extract, 10.92 % ash, and 312 1ME (kcal/kg) (Nworgu, 2007).

The seeds can be consumed by roasting or boiling and are sometimes used as soup thickeners (Kuku *et al*., 2014). The seeds contain 13 % oil, which can be used for cooking, marmalade manufacturing and cookie formulations (Kuku *et al*., 2014). The oil of *T. occidentalis* seeds has high iodine and a high content of unsaturated fatty acids when compared to palm oil. The seed oil is also suitable for manufacturing of soaps, paints and vanishings. The fermented seeds of fluted pumpkin are used in the production of “ogiri ugu”, locally made custard. The seeds possess nutritive and calorific values, which make them useful as foods and good sources of edible oils and fats (Eddy *et al.*, 2011; Odoemelam, 2005). Kuku *et al*. (2014) reported that fluted pumpkin seeds are rich in essential nutrients but the crude protein content of the processed seed (28.09 %) was higher than that of the unprocessed seed (26.93 %). Seed residue after oil extraction is used as animal feeds (Akang *et al*., 2011). Fluted pumpkin

seeds have been reported to be rich in proteins (Kuku *et al*., 2014). Dietary incorporation of the seeds of *T. occidentalis* resulted in good growth (Ejike *et al.*, 2010). *T. occidentalis* seed oil has also been tested for use in prophylactic medicine for the alleviation of infertility (Akang *et al*., 2011).

There is, however, a major limitation in the utilization of *T. occidentalis* seeds because they contain antinutritional factors such as enzyme inhibitors, allergens, lectins, and other naturally occurring substances that may influence diet intake, digestibility, absorption and metabolic processes in animals and humans. Previous research reported that the existence of these anti-nutritional factors in food causes growth inhibition as well as digestive and histological perturbations in laboratory models (Chunmei *et al*., 2010).

2.1.3 Moringa seeds

Moringa oleifera (moringa) is also known as 'drumstick tree' or 'horse radish, is the cultivated species of the genus moringa of the family Moringaceae. Several health benefits were reported because of supplementation with moringa leaves or seeds or their extract (Parikh *et al*., 2014). Moringa improved nutrition, boosted food security, fostered rural development support sustainable land care, and foraged for livestock (Al-Malki and El Rabey, 2015). Moringa ameliorated liver fibrosis in rats and reduces liver damage and symptoms of liver fibrosis, decreased the CCl_4 induced elevation of serum aminotransferase activities and globulin level, and reduced the elevated hepatic hydroxyproline content and myeloperoxidase activity (Al-Malki and El Rabey, 2015). Parikh *et al*. (2014) reported that the seed length, width and thickness are 8.48 mm, 7.80 mm and 6.41 mm respectively.

Previous proximate composition of moringa seeds has showed high levels of lipids and protein. Alessandro *et al*. (2016) reported that moringa seeds contain protein content, on

average 31.4 %, whereas carbohydrate, fibre and ash contents are 18.4 %, 7.3 % and 6.2 %, respectively. Abdulkarim *et al.* (2005) have described high levels of total proteins in moringa seeds, which turned out to be greater than important leguminous seeds (18 % to 25 % of protein) (Parikh *et al*., 2014). Thus, the defatted seeds of moringa could provide an economical source of protein for use as a food supplement to traditional diets to increase protein intake. Furthermore, the protein content of moringa seeds has a high content of methionine and cysteine, close to that reported for milk and eggs (Alessandro *et al*., 2016). Therefore, they can be consumed together with legumes, which are deficient in sulphur amino acids. Moreover, moringa seeds seem to be free of trypsin inhibitor and urease activity, confirming the high protein digestibility (93 %) of moringa seeds (Oliveira *et al*., 1999; Santos *et al*., 2005). The variation of proximate composition of moringa seeds may be explained by different climatic conditions, time of the year and different soil types from which the seeds were collected (Ferreira *et al*., 2008).

Moringa seeds extract has been reported to be anti-inflammatory, anti-spasmodic, anti-hypertensive, anti- tumor, anti- oxidant, anti-pyretic, anti-ulcer, anti-epileptic, diuretic, cholesterol lowering, anti-diabetic and hepatoprotective (Lai *et al*., 2010; Sharma *et al*., 2012 ; Huang *et al*., 2012a).

2.2 Protein

Protein is a macronutrient, which means is one of the important component needed in relatively large amount by the body, it’s an important building blocks of bones, muscles, blood, skin and cartilage. Protein help to build and repair the body tissues, help in growth and maintenance, aids biochemical reactions, help to maintain proper body pH, balancing body fluid, boosting immune system, transport and stored nutrients and it can

as well serve as energy in situation like fasting, inadequate calorie intake and exhaustive exercise (Gavin, 2018).

Unlike fat and carbohydrate, protein is not store up by the body and therefore has no reservoir to drawn on when needed except to be consume from diet. Protein is needed in a modest amount by the body to function properly. According to the United States, department of health and human services, teenage boys and active men need a total of seven ounces of protein per day, age two to six children, most women and older people need five ounces daily while older children, teenage girls, active women need a total of six ounce per day (Sharma *et al*., 2012).

2.3 Carbohydrate

Carbohydrates are biomolecules whose primary function is to provide the body with energy. Carbohydrate in diet are digested and converted to glucose which will be taken up by the body cells to produce fuel molecules called adenosine triphosphate (ATP). Cell uses this ATP to power variety of metabolic tasks some of which are muscles preservation, promote digestive health thereby reducing straining and pains associated with bowel movement, it's also help to alleviate constipation, protection against digestive tract disease. They are found in food like fruits, vegetables, skin and seeds o fruits, bread, pasta, diary product and grains (Sharma *et al*., 2012).

2.4 Fat

Dietary fats are essential to give the body energy and support; they protect organs and help in keeping the body warm, cell growth, help in the absorption of some nutrients and produce important hormones. Fat can have effect on the body cholesterol level because they are more energy dense than carbohydrate and proteins (Ferreira *et al*., 2008).

2.5 Minerals

Minerals are very essential for the healthy state of the body, minerals are required for proper functioning of the bone, muscles, heart and brain, they are also required in nerve impulses transmission, maintaining all physiological processes, acting as catalyst for many biological reactions within human body, digestion and utilization of all nutrients in food. Correct balance of minerals helps in proper assimilation of vitamins, for instance, calcium is needed for vitamin C utilization and zinc for vitamin A (Fukuwatari and Shibata, 2008).

Essential minerals are of two kinds, macro minerals and trace minerals, these groups of minerals are equally important, but trace minerals are needed in smaller amounts than the macro minerals. Essential minerals are usually provided by a balanced diet. The two tables, 2.1 and 2.2 listed minerals, what they do in the body (their functions), and their sources in food (Fukuwatari and Shibata, 2008).

Table 2.1: Function and Sources of Macro Minerals

Mineral	Function	Sources
Sodium	Needed for proper fluid balance, nerve transmission, and muscle contraction	Table salt, soy sauce; large amounts in processed foods; small amounts in milk, breads, vegetables, and unprocessed meats
Chloride	Needed for proper fluid balance, stomach acid	Table salt, soy sauce; large amounts in processed foods; small amounts in milk, meats, breads, and vegetables
Potassium	Needed for proper fluid balance, nerve transmission, and muscle contraction	Meats, milk, fresh fruits and vegetables, whole grains, legumes
Calcium	Important for healthy bones and teeth; helps muscles relax and contract; important in nerve functioning, blood clotting, blood pressure regulation, immune system health	Milk and milk products; canned fish with bones (salmon, sardines); fortified tofu and fortified soy milk; greens (broccoli, mustard greens); legumes
Phosphorus	Important for healthy bones and teeth; found in every cell; part of the system that maintains acid-base balance	Meat, fish, poultry, eggs, milk, processed foods (including soda pop)
Magnesium	Found in bones; needed for making protein, muscle contraction, nerve transmission, immune system health	Nuts and seeds; legumes; leafy, green vegetables; seafood; chocolate; artichokes; "hard" drinking water
Sulfur	Found in protein molecules	Occurs in foods as part of protein: meats, poultry, fish, eggs, milk, legumes, nuts

Source: Fukuwatari and Shibata (2008)

Table 2.2: Function and Sources of Trace Minerals (Micro Minerals)

Mineral	Function	Sources
Iron	Part of a molecule (hemoglobin) found in red blood cells that carries oxygen in the body; needed for energy metabolism	Organ meats; red meats; fish; poultry; shellfish (especially clams); egg yolks; legumes; dried fruits; dark, leafy greens; iron-enriched breads and cereals; and fortified cereals
Zinc	Part of many enzymes; needed for making protein and genetic material; has a function in taste perception, wound healing, normal fetal development, production of sperm, normal growth and sexual maturation, immune system health	Meats, fish, poultry, leavened whole grains, vegetables
Iodine	Found in thyroid hormone, which helps regulate growth, development, and metabolism	Seafood, foods grown in iodine-rich soil, iodized salt, bread, dairy products
Selenium	Antioxidant	Meats, seafood, grains
Copper	Part of many enzymes; needed for iron metabolism	Legumes, nuts and seeds, whole grains, organ meats, drinking water
Manganese	Part of many enzymes	Widespread in foods, especially plant foods
Fluoride	Involved in formation of bones and teeth; helps prevent tooth decay	Drinking water (either fluoridated or naturally containing fluoride), fish, and most teas
Chromium	Works closely with insulin to regulate blood sugar (glucose) levels	Unrefined foods, especially liver, brewer's yeast, whole grains, nuts, cheeses
Molybdenum	Part of some enzymes	Legumes; breads and grains; leafy greens; leafy, green vegetables; milk; liver

Source: Fukuwatari and Shibata (2008)

2.6 Vitamins

A vitamin is an organic molecule that is an essential micronutrient which an organism needs in small quantities for the proper functioning of its metabolism. Vitamins are essential for the normal growth and development of a multicellular organism, they also enable a multicellular organisms to efficiently use chemical energy provided by food it eats, and to help process the proteins, carbohydrates, and fats required for cellular respiration (Bender, 2003). They can be classified as either water-soluble or fat-soluble vitamins. Water-soluble vitamins dissolve easily in water and, in general, are readily excreted from the body, to the degree that urinary output is a strong predictor of vitamin consumption while fat-soluble vitamins are absorbed through the intestinal tract with the help of lipids (fats) (Fukuwatari and Shibata, 2008). Deficiencies of vitamins can either be primary or secondary. A primary deficiency occurs when an organism does not get enough of the vitamin in its food. A secondary deficiency may be due to an underlying disorder that prevents or limits the absorption or use of the vitamin, due to a "lifestyle factor", such as smoking, excessive alcohol consumption, or the use of medications that interfere with the absorption or use of the vitamin (Wendt, 2015). Vitamin deficiencies involve thiamine (beriberi), niacin (pellagra), vitamin C (scurvy), folate (neural tube defects) and vitamin D (rickets) (Price, 2015).

Table 2.3: Function and Sources of Water Soluble Vitamins

Nutrient	Function	Sources
Thiamine (vitamin B1)	Part of an enzyme needed for energy metabolism; important to nerve function	Found in all nutritious foods in moderate amounts: pork, whole-grain or enriched breads and cereals, legumes, nuts and seeds
Riboflavin(vitamin B2)	Part of an enzyme needed for energy metabolism; important for normal vision and skin health	Milk and milk products; leafy green vegetables; whole-grain, enriched breads and cereals
Niacin (vitamin B3)	Part of an enzyme needed for energy metabolism; important for nervous system, digestive system, and skin health	Meat, poultry, fish, whole-grain or enriched breads and cereals, vegetables (especially mushrooms, asparagus, and leafy green vegetables), peanut butter
Pantothenic acid	Part of an enzyme needed for energy metabolism	Widespread in foods
Biotin	Part of an enzyme needed for energy metabolism	Widespread in foods; also produced in intestinal tract by bacteria
Pyridoxine (vitamin B6)	Part of an enzyme needed for protein metabolism; helps make blood cells	Meat, fish, poultry, vegetables, fruits
Folic acid	Part of an enzyme needed for making DNA and new cells, especially red blood cells	Leafy green vegetables and legumes, seeds, orange juice, and liver; now added to most refined grains
Cobalamin (vitamin B12)	Part of an enzyme needed for making new cells; important to nerve function	Meat, poultry, fish, seafood, eggs, milk and milk products; not found in plant foods
Ascorbic acid (vitamin C)	Antioxidant; part of an enzyme needed for protein metabolism; important for immune system health; aids in iron absorption	Found only in fruits and vegetables, especially citrus fruits, vegetables in the cabbage family, cantaloupe, strawberries, peppers, tomatoes, potatoes, lettuce, papayas, mangoes, kiwifruit

Source: Bender (2003)

Table 2.4: Function and Sources of Fat Soluble Vitamins

Nutrient	Function	Sources
Vitamin J	Needed for proper absorption of calcium; stored in bones	Egg yolks, liver, fatty fish, fortified milk, fortified margarine. When exposed to sunlight, the skin can make vitamin D.
Vitamin E	Antioxidant; protects cell walls	Polyunsaturated plant oils (soybean, corn, cottonseed, safflower); leafy green vegetables; wheat germ; whole-grain products; liver; egg yolks; nuts and seeds
Vitamin K	Needed for proper blood clotting	Leafy green vegetables such as kale, collard greens, and spinach; green vegetables such as broccoli, Brussels sprouts, and asparagus; also produced intestinal tract by bacteria

Source: Bender (2003)

2.7 Antinutritional Factors in Foods

Antinutrients are plant compounds that reduce the body's ability to absorb essential nutrients, they are either natural or synthetic compounds found in a variety of foods especially grains, beans, legumes and nuts which interfere with the absorption of vitamins, minerals and other nutrients, this is because they get in the way of the digestive enzymes, which are key for proper absorption (Habtamu and Negussie, 2014). Nutrition studies focuses on those antinutrients commonly found in food sources and beverages and are found at some level in almost all foods for a variety of reasons. Different types of antinutrients are found in seed, foods contain antinutrients like phytic acid, leptins and saponins naturally. However, their levels are reduced in modern crops, probably as an outcome of the process of domestication (Geo, 2008). Plants use antinutrients as defence mechanism, also help to repel pests, bugs and other predators so the seeds are able to live on and reproduce. These antinutrients are saponins, phytate, oxalate, cyanogenic glycosides and tannins.

Most of the antinutrients in foods are found in the skin. Since many antinutrients are water-soluble, they simply dissolve when foods are soaked. Soaking, sprouting, boiling, fermentation and heating decrease phytate, protease inhibitors, lectins, tannins and calcium oxalate.

2.7.1 Saponins

These are group of naturally occurring oily glycosides, they are known by their bitter or astringent taste, foaming properties and their hemolytic effect on red blood cells, they are found primarily in variety of plants (Habtamu and Negussie, 2014). Saponins have been, and sometimes still are, used as cleaning agents and as foam producers, notably in fire-extinguishing fluids and are useful unprocessed material for steroid hormones

synthesis, pharmacological and medicinal properties, haemolytic properties as well as antimicrobial, insecticidal and mollusicidal activities (Podolak *et al.,* 2010). Similar to lectins, saponins affect the gastrointestinal lining, contributing to leaky gut syndrome and autoimmune disorders. They are particularly resistant to digestion by humans and have the ability to enter the bloodstream and trigger immune responses.

Previous researches by Sun *et al.* (2009) and Spang *et al.* (2004) reveal that a high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans and an antidote against acute lead poisoning and that they possess hypocholesterolemic, immune stimulatory, and anticarcinogenic properties. Saponins are used as adjuvants in viral and bacterial vaccine applications.

2.7.2 Phytate

Phytic acid are usually found in the hulls of nuts, seeds and grains and it has a strong binding affinity to minerals such as calcium, phosphorus, magnesium, iron, copper, and zinc leading to precipitation, thereby making the minerals unavailable for absorption in the intestines by so doing raise the risk of anemia and bone loss (Ekholm *et al.,* 2003; Cheryan and Rackis, 1980). Phytate also inhibits certain essential digestive enzymes like amylase, trypsin and pepsin. Amaylase breaks down starch, while both pepsin and trypsin are needed to break down protein (Habtamu and Negussie, 2014).

2.7.3 Oxalate

They are present in many plants and bind to calcium and prevent its absorption in the human body. The presence of oxalate makes plant (especially legumes) proteins of "poor quality and high consumption of oxalate diet increase renal absorption of calcium, it's also binds with nutrients during digestion in the gastrointestinal tract, rendering them inaccessible to the body, nutritional deficiencies are also likely to occur, as well as severe irritation to the lining of the gut. Oxalic acid binds with calcium, iron and

magnesium, rendering these minerals unavailable to animals (Noonan and Savage, 1999).

2.7.4 Tannins

These are enzyme inhibitor that prevent adequate digestion and can cause protein deficiency and gastrointestinal problems; they decrease protein quality by decreasing digestibility and palatability. Its decrease digestibility by making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen (Felix and D'Mello, 2000) thereby leading to bloating, diarrhea, constipation and other GI issues. (Osagie, 1998) reveal that tannins inhibit the activities of trypsin, chymotrypsin, amylase and lipase and interfere with dietary iron absorption, its decrease feed intake, growth rate and feed efficiency, it's also cause damage of intestinal tract, toxicity of tannins absorbed from the gut, and interference with the absorption of iron and possible carcinogenic effect, high concentrations of tannins in diet have also been reported to depress microbial activities.

2.7.5 Cyanogenic glycoside

Cyanogenic glycosides are derived from five protein amino acids which are leucine, Isoleucine, valine, phenylalanine and tyrosine and from non proteinogenic amino acid cyclopentenyl glycine. The consumption of foods containing cyanogenic glycoside could result in acute or chronic cyanide toxicity, this is because glycosides which, upon hydrolysis, liberate hydrogen cyanide and cyanide molecules, potent toxic anti-nutritional compounds. Therefore, the cyanogenic content release during hydrolysis is potentially deleterious to health (Kuti and Konuru, 2006). Cyanide is an inhibitor of cytochrome oxidase and interferes with aerobic respiratory system. Cooking, as well as other heat treatments hydrolyze the cyanogenic glycosides, minimizing the risk of toxicity (Kuti and Konuru, 2006).

2.8 Some Methods of Food Processing

Food processing is the transformation of unprocessed animal, vegetable, or marine materials by physical or chemical means into tasty, nutritious, and safe food products. Its combine unprocessed food ingredients to produce marketable food products (Johns, 2015). Food is processed for safety, removing toxin, preservation, easing marketing and distribution tasks, and increasing food consistency. In addition, it increases yearly availability of many foods, enables transportation of delicate perishable foods across long distances and makes many kinds of foods safe to eat by de-activating spoilage and pathogenic microorganism (Laudan, 2010). It can also reduce the incidence of food borne disease.

Food processing and preparation activities cover three main fields, which are

(1) The preservation of foods by

(a) Modern methods such as refrigeration, canning and irradiation.

(b) Traditional methods such as drying, salting, smoking and fermentation.

(2) The development of protein rich foods

(62) Food additives.

Methods of processing are curing, drying, fermentation, irradiation, boiling, roasting, freezing and refrigerating, pasteurization and genetic engineering. However, the ones to be considered in this research work are the traditional methods, which are germination, boiling and soaking.

2.8.1 Boiling

Boiling is the method of cooking food in boiling water, or other water-based liquids such as stock or milk. Simmering is gentle boiling, while in poaching the cooking liquid

moves but scarcely bubbles. Water has a boiling point of 100 °C or 212 °F. The boiling point of the liquid can be alter by pressure and a change in composition of the liquid, thus, high elevation cooking, generally takes longer since boiling point is a function of atmospheric pressure. Boiling helps in killing microbes, boiling, can degrade antinutrients like lectins, tannins and protease inhibitors. Previous studies show that boiling pigeon peas for 80 minutes reduced protease inhibitors by 70 %, lectin by 79 % and tannin by 69 % but phytate is heat-resistant and not as easily degraded with boiling (Osman, 2007; Vijayakumari *et al.,* 1995). The cooking time required is dependent on the type of antinutrient, food plant and the cooking method. Generally, a longer cooking time results in greater reductions of antinutrients (Vijayakumari *et al.,* 1995).

2.8.2 Soaking

Soaking have been used to process foods, reducing levels of antinutrients and increasing nutrient quality of foods. Soaking oats followed by sprouting the oats reduces phytate content and doubles the amount of absorbed zinc in comparison with untreated oats. Zinc content is improved when leavened products are used (Ikeda and Murakamai, 1995). Soaking help to reduce the viscosity and activate endogenous phytases that break down phytate into lower inositol phosphates, decrease protease inhibitors, lectins, tannins and calcium oxalate. It also improve flavor digestibility of the products and increase the content of vitamin and minerals, and increase the B-complex vitamins. It helps to break down the higher carbohydrates and other storage molecules such as calcium, magnesium, and phytate because it enhances enzymatic hydrolysis of phytates (Akpapunam and Sefa-Dedeh, 1997).

2.8.3 Sprouting (germination)

Germination is a natural process and a period in the life cycle of plants when they start emerging from the seed. During germination, water diffuses through the seed coats into

the embryo, which has been almost completely dry during the period of dormancy, causing a swelling of the seed; the swelling is often so great that the seed coat is ruptured. With the absorption of oxygen by the seed, energy is made available for growth (Romo-parade *et al.,* 1985). The foodstuffs stored in the endosperm or in the cotyledons are broken down by enzymes into simpler substances that are transported through the embryo to its difference parts. During sprouting, changes take place within the seed that lead to the degradation of antinutrients such as phytate by 37 % - 81 % and protease inhibitors in various types of grains and legumes (Sattar *et al.,* 1990).

2.9 Functional Properties of Foods

The functional properties of food are the physical changes, chemical changes and organoleptic properties that reflect the complex interaction between the composition, structure, molecular conformation and physico-chemical properties of a food that occur during food storage, preparation and presentation together with the nature of environment in which these are associated and measured (Kaur and Singh, 2006; Siddiq *et al.,* 2009). Food functional property helps in characterizing the structure, quality, nutritional value, and acceptability of a food production. Examples of a functional property may include solubility, water absorption, water retention, frothing ability, elasticity, and absorptive capacity for fats and foreign particles. Functional characteristics are needed to enumerate and possibly help to predict how new proteins, fat, fibre and carbohydrates may behave in a particular system as well as demonstrate whether or not such protein can be utilized to stimulate or replace conventional protein (Kaur and Singh, 2006; Siddiq *et al.,* 2009). Awuchi *et al*. (2019) reported that functional properties of foods and flours are influenced by the components of the food material, especially the carbohydrates, proteins, fats and oils, moisture, fibre, ash, and other ingredients or food additives added to the food (flour), such as sugar alcohols.

Functional foods can be designed to have physiological benefits and to reduce the risk of chronic disease beyond basic nutritional functions, and may be similar in appearance to conventional food and consumed as part of a regular diet (Siddiq *et al.,* 2009). Usual functional properties constitute emulsification, hydration, viscosity, foaming, solubility, gelation, cohesion and adhesion.

2.9.1 Water and oil absorption capacity

Water and oil absorption capacity help to determine flour emulsification and binding abilities to high fat food.

2.9.1.1 *Water absorption capacity*

Water absorption capacity also known as water hydration, this is the amount of water taken up by flour to achieve the desired consistency or optimal end result and create quality food product. It is usually defined based on flour weight. It shows how hydrophilic flour can be and therefore would be a useful ingredient in viscous food (Oyeleke *et al*., 2012). Very low or excessive water absorption can negatively affect the quality of food products. Water absorption is usually determined by the weight of the food or flour. For instance, 55 % water absorption means 55 lbs of water is required for every 100 lbs of flour. When water and flour are mixed, hydration of gluten-forming proteins (glutenin and gliadin), as well as the damaged starch and other ingredients occurs, this hydration process is achieved when starch and protein molecules create hydrophilic interactions and hydrogen bonds with the molecules of water.

Carson (2017) reported that water absorption capacity vary from 50-54 % in a cookie formula, 60-62 % in a standard white bread formula, and sprouting flour cause increase in enzymatic activity in the flour which in turn increase water absorption and Maillard reaction. Water absorption varies among tested samples and this may be attributed to the nature and types of polar group in the samples (Hassan and Abou- arab, 1993).

Increased in WAC value can be attributed to many hydrophilic components contained in foods such as carbohydrate (especially polysaccharides), proteins, especially polar amino acid residues. This have high affinity for water molecules (Sreerama *et al.,* 2012), increase in the amylose solubility and leaching as well as loss of the crystalline structure of starch and this increase suggests that the combination of different flours can be used in formulation of many foods such as processed cheese, bakery products, sausage, and dough. While decrease in water absorption in some flours may be as a result of less availability of polar amino acids in the flours (Iwe *et al.*, 2016).

2.9.1.2 *Oil absorption capacity*

Oil absorption capacity is the binding of fat by the non-polar side chain of proteins. It's an essential functional property that contributes to enhancing mouth feel while retaining the food products' flavour (Iwe *et al.,* 2016). Its help in determining the flavour retention in food material (Oyeleke *et al*., 2012), due to these properties, the flours with good OAC are used as functional ingredient in foods such as sausages, whipped toppings, angel and sponge cakes, chiffon dessert etc. These properties are due to the physical entrapment of oil, it is also related to the number of non-polar side chains on protein that binds hydrocarbon chains on the fatty acids (Hassan and Abou- arab, 1993). The oil and water binding capacity of protein in food depend on the intrinsic factors like protein conformation, amino acid composition and surface polarity or hydrophobicity (Suresh and Samsher, 2013).

2.9.2 Emulsion capacity and stability

Emulsion is a mixture of two or more liquids that are normally immiscible (unmixable or unblendable) thus forming a dispersed (one liquid) and continuous phase (the other liquid phase) with the boundary between the phases called the interface. Emulsion

capacity (EC), of flour is associated with the amount of oil, non-polar amino acids residues on the surface of protein, water, and other components in the food. An increased number of non-polar amino acids residues on the surface of protein will reduce the energy barrier to adsorptions which depends on the protein structure.

Emulsion stability refers to the ability of an emulsion to resist change in its properties over time; the stability of emulsions can be characterized using techniques such as light scattering, focused beam reflectance measurement, centrifugation, and rheology. Emulsion stability is important for stabilization of additives in production of food (Oyeleke *et al*., 2012). Oil-in-water emulsions can be used for food products such as crème, mayonnaise, hollandaise sauces, homogenized milk and vinaigrette while water-in-oil emulsions are less common in food, but still exist and can be used in preparation of Butter and Margarine (Oyeleke *et al*., 2012).

2.9.3 Foam capacity and stability

Foam capacity show how flour can be used as a whipping or aerating agent in food system (Oyeleke *et al*., 2012), is measured as the amount of interfacial area created by whipping the food or flour while foam stability refers to the ability of food to stabilize against mechanical and gravitational stresses and is a measured of the time require to lose either 50 percent of the liquid or 50 percent of the volume from the foam (Awuchi *et al*., 2019). Protein is mainly responsible for foaming. Foaming capacity and stability always have inverse relationship and they generally depend on the interfacial film formed by the proteins, which maintains the suspension of air bubbles and slows down the coalescence rate (Awuchi *et al*., 2019).

2.9.4 Bulk density

Bulk density of food depends on combined effect of interested factors such as attractive interparticle forces, particle size, numbers of contact points and it is not an intrinsic property of a food material (Hassan and Abou- arab, 1993), a change in any one of the flour characteristics may result in significant change in the flour bulk density (Hassan and Abou- arab, 1993). Bulk density reflects the relative volume or capacity of the required packaging material. The higher the bulk density of the flour, the denser the packaging material required for packaging. It indicates the porosity of a food product which impacts the design of the package and can be used in determining the type of the required packaging material (Iwe *et al.*, 2016). Spray dried particles usually have rise bulk density when compare to freeze-dried because spray-dried samples yield denser product (Oyeleke *et al*., 2012). Hassan and Abou-arab (1993) revealed that a change in bulk density might result from moisture absorption, chemical reactions or mechanical attrition. Variation in bulk density of flours could be as a result of variation in starch content of the flours. The higher the starch content the more likely the increase in bulk density since starch forms the main structure and bulk of many food products e.g. in biscuits, bread, cakes, and pastries. Also, bulk density depends on factors such as geometry, method of measurement, particle size, surface properties, and solid density of the materials and can be improved when the particles are smaller, properly tapped/vibrated, compactable, and with a suitable packaging material (Iwe *et al.*, 2016). High bulk density of flours suggests their suitability for application in food preparations. On the other hand, low bulk density would be useful in the formulation of complementary foods (Suresh and Samsher, 2013).

2.9.5 Swelling capacity (SC)

The swelling capacity is a measure of the ability of starch to absorb water and swell, and also shows the extent of associative forces in the starch granules. SC is considered a quality measure in some food products such as bakery products. It is an indication of the non-covalent bonding between the molecules of starch granules and also one of the factors of the α-amylose and amylopectin ratios (Iwe *et al.,* 2016). Suresh and Samsher (2013) reported that SC of flours are Influenced by the particle size, species variety and method of processing or unit operations. Swelling capacity increases with increase starch content of foods and flours and this explains why different flours from different (plant) sources and species have different swelling capacities (Awuchi *et al.,* 2019).

2.9.6 Solubility

Solubility (in food system) can be define as a chemical and functional property referring to the ability of a given food substance to dissolve in a solvent, usually water or oil. It is measured and determined in terms of the maximum quantity of solute dissolved in a given solvent at equilibrium (Awuchi *et al.,* 2019).

Substance solubility depends fundamentally on the physical and chemical properties of the solvent, solute, pressure, pH, temperature, and presence of other chemicals in the solution. The extent of the solubility of a flour substance in a specific solvent is commonly measured as the saturation concentration, in which addition of more solute does not have effect on the concentration of the solution and rather starts to precipitate the excess quantity of solute. Opong *et al*., (2015) reported that the lipids reduce water absorption capacity of flours which can lead to reduced swelling capacity and consequently reduced solubility, so therefore, increase in food digestibility can be seen in food with high solubility and this suggest excellent use of the food for infant formula and food and it's also useful and advantageous when separating mixtures. Solubility of

food substances can be affected by temperature, pressure, polarity and concentration of a solvent (Ammar *et al.,* 2016).

2.10 Haematological Parameters

Haematological parameters are those parameters that are related to the blood and blood forming organs and are important indicators of health status in animals, besides, they have been an indispensable tool in the diagnosis, treatment and prognosis of many diseases (Waugh *et al.,* 2001). Blood examination creates avenue to investigate the presence of several metabolites and other constituents in the body of animals and how it plays a vital role in the physiological, nutrition and pathological status of an organism (Doyle, 2006) and can provide useful information for the diagnosis and prognosis of diseases in animals.

Blood constituents change in relation to physiological conditions of health. Animals with good blood composition show better performance (Isaac *et al*., 2013). Examining blood for their constituents can also provide information for diagnosis and prognosis of diseases in animals. Haematological parameters include, red blood cells, white blood cells, mean corpuscular volume (MCV), mean corpuscular haemoglobin and Mean corpuscular haemoglobin concentration (MCHC) (Oyawoye and Ogunkunle, 2004).

2.10.1 Red blood cell

Red blood cells also called erythrocytes, its serve as a carrier of oxygen to various tissues (Vinay *et al,* 2007). The erythrocytes cytoplasm is rich in hemoglobin (an iron-containing biomolecule that can bind oxygen and is responsible for the red colour of the cells and the blood) and It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Chineke *et al.,* 2006). Mature red blood cells are flexible and oval biconcave in shape in human. According to Isaac *et al*. (2013) red blood cell is involved in the transport of oxygen and carbon dioxide in the

body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al.,* 2013; Isaac *et al.,* 2013).

According to the Leukemia & Lymphoma Society, the normal red blood cell range for men, women and children ranges from 4.7 to 6.1 million cells per microliter (mcl), 4.2 to 5.4 million mcl and 4.0 to 5.5 million mcl respectively. Low red blood cells counts may indicate anaemia as a result of blood loss, bone marrow suppression, and malnutrition such as iron deficiency, over-hydration, or mechanical damage to red blood cells. Abnormally high red blood cells counts may indicate congenital heart disease, some lung diseases, dehydration, kidney disease or polycythaemia (Yazdanbakhsh *et al.,* 2000).

Red blood cell indices are hemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Hemoglobin is the protein molecules and oxygen-carrying pigment of red cells, it plays an important role in maintaining the shape of the red blood cells. There are millions of hemoglobin molecules in each red blood cell. Decreases in hemoglobin occur for the same reasons as decreased Red blood cells (Aghara, 2014). The packed cell volume (PCV) measures the volume of cells as a percentage of the total volume of cells and plasma in whole blood. This percentage is usually three times greater than the hemoglobin. The measurement depends on the number and size of red blood cells. It's normal range for men is 40.7 %–50.3 % while for women is 36.1 % – 44.3 % PCV levels serve as an indicator of health conditions, after hemorrhage or excessive intravenous fluid infusion the PCV will be low (Bren *et al*., 2015).

The mean corpuscular volume, or “mean cell volume” (MCV), is a measure of the average red blood cell volume (i.e. size) that is reported as part of a standard complete blood count. In patients with anemia, it is the MCV measurement that allows classification as either a microcytic anemia (MCV below normal range) or macrocytic anemia (MCV above normal range). It is calculated (in litres) by dividing the hematocrit by the red blood cell count (number of red blood cells per litre). The results are typically reported in femtolitres. The normal referance range is typically 80-100 fL. In pernicious anemia (macrocytic), MCV can range up to 150 femtolitres. Vitamin B_{12} and/or Folic acid deficiency has also been associated with macrocytic anemia (high MCV numbers) (Bamishaiye *et al.*, 2010). Low MCV values indicate the cells are microcytic (small cells) and are often evident with conditions such as iron deficiency, lead poisoning, thalassemias and chronic diseases (Corbett, 1996).

The mean corpuscular hemoglobin, or “mean cell hemoglobin” (MCH), is the average of weight of hemoglobin per red blood cell in a blood sample. It is reported as part of a standard complete blood count: MCH value is diminished in hypochromic anemias (Aster, 2004).

The mean corpuscular hemoglobin concentration, or MCHC, is a measure of the concentration of hemoglobin in a given volume of packed red blood cell. It is reported as part of a standard complete blood count. It is calculated by dividing the hemoglobin by the hematocrit or from directly measured hemoglobin and is the RBC index that is provided on routine hemograms. Reference ranges for blood tests are 32 to 36 g/dl, or between 4.9 to 5.5 mmol/L. It is diminished (hypochromic) in microcytic anemias, and normal (normochromic) in macrocytic anemias. This is the most sensitive test for iron deficiency anemia (Aster, 2004).

2.10.2 White blood cell

White blood cell (leukocyte) is the cellular component of the blood that have a nucleus but lacks hemoglobin, white blood cell and its differentials help to defend the body against infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response (Soetan *et al*., 2013). White cells are highly differentiated for their specialized functions, and they do not undergo cell division in the bloodstream; however, some retain the capability of mitosis. There are five major types of white blood cells, they are neutrophils, lymphocytes, eosinophils, monocytes, basophils (Soetan *et al*., 2013). Normal range or WBC in infant, under 2 years old children and over 2years to adult are 9,000 to 30,000, 6,200 to 17,000 and 5,000 to 10,000 respectively. Symptoms of abnormal WBC are body aches, fever, chills, and headaches.

2.10.2.1 *Lymphocytes*

Lymphocytes are body's main types of immune cells. They are made in the bone marrow and found in the blood and lymph tissue. Lymphocytes are further divided into B cells, T cells and natural killer cells (Cooper, 2015). Berrington *et al.* (2005), reported that T cells are involved in cell-mediated immunity, whereas B cells are primarily responsible for humoral immunity (relating to antibodies), they both perform the function of identifying a specific non-self-antigen during antigen presentation after which generate specific responses by producing large quantities of antibodies for defense, produce cytokines (for immune response direction) and toxic granules (which contain powerful enzymes to induce the death of pathogen-infected cells). Natural killer cells are innate immune system which help in defending the host from tumors and virally infected cells, they are activated in response to interferon (Abbas *et al.*, 2003).

2.10.2.2 *Monocytes*

Monocytes are the largest type of white blood cells. They produce substances which have defensive functions such as lysozyme, interferons and other substances which modulate the functionality of other cells. Macrophages cooperate in the immune defense. They reveal molecules of digested bodies on the membrane and present them to more specialized cells, such as B and T lymphocytes.

The monocytes are therefore phagocytic cells that remove foreign materials such as injured and dead cells, microorganisms and other particles from the site of injury, particularly during viral or bacterial infections. Normal levels, range from 2 % - 8 % to 4 % - 10 % (Ziegler-Heitbrock, 2007).

2.10.2.3 *Eosinophils*

Eosinophils play a significant role in the inflammation related to allergies, eczema, and asthma. It isolate and control the immune response at the site of an infection, it also destroy invading germs like viruses, bacteria, or parasites such as hookworms (Bolus *et al*., 2015). Normal blood levels range from 0 % -7 % (Bandeira-Melo *et al*., 2002).

2.10.2.4 *Neutrophils*

Neutrophils have a diameter of 12-15 μm. The function of neutrophils is to destroy and ingest bacteria and fungi. Neutrophils arrive first at the site of inflammation; therefore, their numbers will increase greatly immediately after an injury or during the inflammatory process. Their life span is approximately 10 hours, and then a cycle of replenishing neutrophils must occur. Besides during inflammation, neutrophils increase with such conditions as stress, necrosis from burns and heart attack. Normal levels range from 45 %-74 % (Bamishaiye *et al*., 2010).

2.10.2.5 *Basophils*

Basophils are responsible for inflammatory reactions during immune response. They produce compounds that co-ordinate immune responses, like histamine and serotonin which induce inflammation, heparin (that prevents blood clotting) (Stone *et al.,* 2010). Basophils play an important role in parasitic infections and allergies and normal blood levels range from 0 % -2 % (Voehringer, 2009).

2.10.3 Platelet

Platelet also known as thrombocytes form about 20 % of the red blood cells, it have no cell nucleus it is a segment of cytoplasm, it major function is to contribute to hematostasis that is, responsible for blood clotting. They do so by releasing compounds like serotonin which reduce the diameter of lesion vessels and slow down the blood flux, the fibrin which trap cells and forms the clotting. A normal platelet count in a healthy individual is between 150,000 and 450,000 per microlitre of blood (150–

23) $\times 10^9$/L). Low platelet concentrations may suggest that the process of clot formation may be prolonged resulting in excessive loss of blood in the case of injury (Machlus *et al.,* 2014).

2.11 Liver Function Tests

Liver function tests are groups of blood tests that measure different enzymes, proteins, and other substances made by the liver, it help in diagnosis, monitoring therapy and assessing prognosis of the liver. It also gives information about the liver state of health. Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction (Mc Clatchey, 2002). These tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and monitor the response to treatment. These tests

include prothrombin time (PT/INR), activated partial thromboplastin time (aPTT), albumin, bilirubin (direct and indirect), the liver transaminases [aspartate transaminase (AST) , alanine transaminase (ALT) and alkaline phosphatase (ALP)] which are routinely used to examine its functionality. These enzymes measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a bio-marker of hepatocyte injury. Alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) serve as markers of biliary function and cholestasis (Yakubu *et al.*, 2007) and high levels in the blood could suggest liver damage (Giannini *et al*., 2005).

2.11.1 Total plasma proteins

Blood contains albumin and globulin, albumin proteins keep fluid from leaking out of your blood vessels. Globulin proteins play an important role in your immune system (Sana *et al.,* 2016). The total plasma proteins are albumin, α_1- globulins, α_2- globulins, β –globulins and γ-globulins, liver synthesize many plasma proteins, few others are synthesized by macrophages. Immunoglobulins are mainly derived from the B cells of the immune system (Sana *et al.*, 2016). Most plasma proteins are taken up by pinocytosis into the capillary endothelial cells or mononuclear phagocytes where they are catabolized (Kasper *et al.,* 2018).

Total protein normal range is between 6 to 8.3 grams per deciliter (g/dl). Raised plasma concentrations may be due to loss of protein free fluid or a major increase in one or more immunoglobulins. Low plasma total protein concentrations may be due to hypoalbuminemia or profound immunoglobulin deficiency (Sana *et al.,* 2016). Reduction in the total plasma concentration may occur in malnutrition or malabsorption. A decrease in total plasma concentration may also be an indication of liver damage

(cirrhosis), although in some chronic liver diseases, albumin level is low, but globulin level is high giving a normal total protein level, reason might be that liver cannot produce albumin, thus the low albumin level, but globulins are mostly made in reticuloendothelial system, thus their levels may increase during infection (Thapa and Walia, 2007).

2.11.2 Albumin

This is a protein made in the liver; it is the main constituent of total protein, it normal range is between 3.5 to 5.3 g/dl. Albumin levels are decreased in chronic liver disease, such as cirrhosis and nephrotic syndrome where it is lost through the urine. Low albumin can result to edema since the intravascular oncotic pressure becomes lower than the extravascular space. Plasma albumin measurements are useful in assessing the chronicity and severity of liver disease. Low concentration of plasma albumin is also seen in patients with protein energy malnutrition. Plasma albumin concentrations have also been used to detect and monitor protein nutritional status. Increased urinary and gastrointestinal loss of albumin also causes of decreased concentration of plasma albumin (Lee and Jacobs, 2005).

2.11.3 Total bilirubin

This a waste product made by the liver, and it include both the unconjugated (indirect) and conjugated (direct) bilirubin. Unconjugated bilirubin is formed as a result of breakdown of heme product. The liver cleans the blood of unconjugated bilirubin, by conjugating it to make it water-soluble through an enzyme named UDP-glucuronyl-transferase. Unconjugated bilirubin increases predominantly as a result of overproduction, hepatic uptake reduction of unconjugated bilirubin and reduction in conjugation of bilirubin (Lisa and VanWagner, 2015). Overproduction can be caused by haematoma reabsorption and ineffectiveness of erythropoiesis leading to increased

destruction of red blood cell (Shivaraj *et al*., 2009). Increase in conjugated bilirubin is directly proportional to the degree of hepatocyte injury and viral hepatitis (Lisa and VanWagner, 2015).

Increased total bilirubin causes a condition known as jaundice, which may occur as a result of increased bilirubin production due haemolytic anaemia and internal hemorrhage. Increased total bilirubin in the plasma may also occur due to liver damage which may result from reduced hepatocyte uptake of bilirubin, impaired conjugation of bilirubin and reduced hepatocyte secretion of bilirubin. Obstruction of the bile duct may also lead to increased plasma bilirubin (Saukkonen *et al*., 2006). Neonates are especially vulnerable to high bilirubin levels due to an immature blood-brain barrier that predisposes them to kernicterus/bilirubin encephalopathy, which can result in permanent neurological damage. Neonates also have a low amount of functional UDP-glucuronyl-transferase and can have elevated unconjugated bilirubin, since conjugation is limited. So, newborns are often treated with blue light (420-470 nm) to turn the hydrophobic, albumin-binding unconjugated bilirubin into a form that is more hydrophilic and able to be secreted in urine, sparing the neonate's brain (Mc Clatchey, 2002).

2.11.4 Aspartate transaminase (AST)

Aspartate transaminase is an enzyme that catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate to form glutamate and oxaloacetate, requiring the coenzyme pyridoxal phosphate. AST is present in high concentrations in liver, followed by heart, muscle, kidney, brain, pancreas, and lungs (Kasper *et al*., 2018). AST is a relatively less specific indicator of liver damage compared to ALT because of its wide range of AST containing organs. In normal conditions, it ranges from 0 – 35 IU/L, so therefore an increase in AST levels above normal can be caused by trauma or surgery

(especially after cardiac surgery) or after a severe haemolytic episode (Shivaraj *et al*., 2009).

2.11.5 Alanine transaminase (ALT)

Alanine transaminase is an enzyme made and found in high concentrations by the liver, it catalyses the reversible transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate (Adeva-Andany *et al.*, 2016).

Found in lesser amount in the kidneys, heart, and muscles. It catalyses the transamination reaction, and only exists in a cytoplasmic form. In normal conditions, it ranges from 7 – 56 IU/L (Shivaraj *et al*., 2009), elevation of this enzymes indicate liver disease. Although serum activities of both AST and ALT become elevated whenever disease processes affect liver cell integrity. ALT is considered to be more specific to the liver than AST, because AST levels may be elevated in cases of cardiac or skeletal muscle injury while ALT is not (Murray *et al.*, 2012). Serum elevations of ALT activity are rarely observed in conditions other than parenchymal liver disease. In most types of liver disease ALT activity is higher than that of AST, exceptions may be observed in hepatic cirrhosis, alcoholic hepatitis and liver neoplasia (Kasper *et al*., 2018).

2.11.6 Alkaline phosphatase (ALP)

ALP is an enzyme that line the biliary ducts of the liver, it catalyses the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates (Saukkonen *et al*., 2006). It is found in liver, bone, small intestine, kidneys and placenta. Elevations in serum ALP activity commonly originate from the liver and bone, hence, used to

detect liver and bone disorders. It ranges from 41 to 133 IU/L in normal condition (Shivaraj *et al.*, 2009). Therefore, elevation in ALP activity in the serum may indicate the presence of hepatobiliary disease (Ramaiah, 2007). An elevated serum ALP activity is also observed in various bone disease like rickets, osteolmalacia and bone cancers. Transient elevations of ALP activity may be found during healing of bone fractures. Physiological bone growth increases bone ALP in the serum, this account for the fact that growing children ALP enzyme concentration is 1.5 to 7 times that of a healthy adult (Adeyemi *et al*., 2015).

2.12 Renal Function Tests

Nephron is the functional unit of the kidney, which consists of the glomerulus, proximal and distal tubules, and collecting duct. The kidneys play a vital role in the excretion of waste products and toxins such as urea, creatinine and uric acid, regulation of extracellular fluid volume, serum osmolality and electrolyte concentrations, as well as the production of hormones like erythropoietin and 1,25 dihydroxyvitamin D and rennin (Okoro and Farate, 2019). Examining the renal function is important in the management of kidney disease or pathologies affecting it (Hounkpatin *et al.*, 2019). Renal function tests have capacity in identifying the presence of renal disease, monitoring the response of kidneys to treatment, and determining the progression of renal disease (Damiati, 2019).

2.12.1 Creatinine

Creatinine is a waste product produced by muscles by the breakdown of a compound called creatine (a compound that produces energy needed to contract muscles). The presence of creatinine in the blood during diagnosis is a tool for kidney function, due to

the fact that high creatinine level in the blood is an indicator of poor clearance of creatinine by the kidneys (Levey *et al.*, 2006).

Creatine is an amino acid produced naturally produced in the human body by the kidney, liver and pancreas. It is synthesized primarily in the liver from the methylation of glycocyamine and then transported through blood to the other organs, muscle, and brain, where it becomes the high-energy compound phosphocreatine through phosphorylation (Taylor, 1989). Creatine conversion to phosphocreatine is catalyzed by creatine kinase; spontaneous formation of creatinine occurs during the reaction (Allen, 2012). Creatinine is removed from the blood mainly in the kidney by glomerular filtration. Deficiency in kidney filtration causes a rise in blood creatinine concentrations. Therefore, creatinine concentrations in blood and urine may be used to calculate the creatinine clearance, which correlates approximately with the glomerular filtration rate (GFR). Blood creatinine concentrations may also be used alone to calculate the estimated glomerular filtration rate. The reference range for serum creatinine ranges from 0.5-1.2 mg/dL or 44-106 Î¼mol/L (SI units) (Pagana *et al.*, 2019). Decreased clearance by kidney results in an increased blood creatinine and this do not necessarily means renal dysfunction, high reading of serum creatinine might be as a result of increased production of creatinine, excessive intake of protein and creatine supplements, also Intense exercise can increase creatinine by increasing muscle breakdown (Samra and Abcar, 2012).

2.12.2 Urea

Urea is protein and amino acid catabolism nitrogenous end product which is predominately cleared by the kidneys from the body, initially, proteins are degraded to constituent amino acids, which are in turn deaminated producing ammonia, which is

toxic (Weiner *et al*., 2015). The biosynthesis of urea from amino acid nitrogen is carried out exclusively by hepatic enzymes of the urea cycle.

Plasma urea concentration reveal the balance between urea production and elimination in the liver and by the kidneys respectively, therefore, accumulation of plasma urea can be caused by increased urea production, decreased urea elimination or by both consequently leading to kidney disease (Weiner *et al.*, 2015). Measurement of blood and plasma urea has been used as an indicator of kidney function, although a number of extra renal factors may influence the circulating urea concentration, limiting its value as attest of kidney function. The factors include a high protein diet, increased protein catabolism, reabsorption of blood proteins after gastrointestinal hemorrhage, dehydration and decreased perfusion of the kidneys (Shivaraj *et al*., 2009).

2.12.3 Electrolytes

Serum electrolytes are very important when assessing the renal system these include calcium, chloride, magnesium, phosphorus, potassium, and sodium. However, the serum concentrations of these electrolytes are variable and do not reflect total body stores. Electrolyte concentrations in the serum are governed by dietary intake, renal excretion and pathological conditions. Serum concentration could serve as indices of for determining state of the kidney (Hasona and Elasbali, 2016).

Chloride (Cl^-) is an extracellular electrolyte and the major anion in serum and is influenced by extracellular concentrations of sodium and bicarbonate (HCO_3^-) and therefore interpretation of serum chloride concentration requires knowledge of serum sodium concentration and consideration of acid–base status. Serum chloride concentration is increased in renal tubular acidosis and primary hyperparathyroidism. It

is decreased by the administration of drugs such as thiazides, loop diuretics, and corticosteroids (Hasona and Elasbali, 2016).

Sodium is an extracellular electrolyte and is the major cation in serum or plasma, it is measured to assess water and sodium balance. Its concentration is controlled in concert with regulation of blood volume and plasma osmolality. Serum sodium concentration is increased in dehydration. It is decreased in Addison's disease and by diuretic therapy, ascites (due to dilution), congestive heart failure, renal insufficiency, and excessive water intake (Nagai *et al.,* 2017).

Potassium (K^+) is an intracellular electrolyte, is the major intracellular cation and is maintained within narrow limits because of its critical role in neuromuscular and cardiac excitability. The serum concentration is sensitive to changes in acid-base status. Serum potassium level is elevated in acidosis, dehydration, and renal insufficiency, and increases in response to administration of some drugs, such as spironolactone. It is decreased in overhydration and alkalosis, and declines with administration of drugs such as corticosteroids, amphotericin, and lithium carbonate (Vairo *et al.,* 2017).

2.13 Lipid Profile Test

Lipid profile is a panel of blood tests that serves as an initial diagnostic tool for abnormalities in lipids. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease and forms of pancreatitis, and other diseases. The lipid profile typically includes: low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides and total cholesterol. Using these values, very low-density lipoprotein (VLDL) and cholesterol: HDL ratio also can be calculated.

The clinical significance of lipids is primarily associated with their contribution to coronary heart disease and various lipoprotein disorders. Numerous studies have established that when the total cholesterol and LDL cholesterol are high, the incidence and prevalence of coronary heart disease are also high. In contrast to LDL cholesterol, increased HDL cholesterol concentrations have been shown to be protective for coronary heart disease in both epidemiological and clinical trial studies. The measurement of plasma lipids and lipoproteins is therefore a valuable means to identify individuals risk coronary heart disease (Koekemoer *et al.*, 2017).

Triglyceride and cholesterol contain small percent of proteins and are lighter in density. In the fasting state most plasma triglyceride are present in VLDL, while in the postprandial state, chylomicrons contribute to the total plasma triglyceride concentration. LDL carries about 70 % of total plasma cholesterol but very little triglyceride, while HDL contains 20 % to 30 % of plasma cholesterol. Chylomicrons transport dietary lipids absorbed in the intestine to the liver and peripheral tissues. VLDL transfers hepatic derived lipid especially triglycerides to peripheral cells for energy metabolism (Mahmood, 2014). LDL cholesterol distributes and delivers cholesterol to peripheral tissues; this is mediated by binding of LDL to specific receptors on the plasma membrane target cells. The number of LDL receptors on the cell membrane depends on the degree of accumulation of intracellular cholesterol, which down regulates transcription of the LDL receptor gene. An increase in LDL cholesterol therefore leads to decrease in LDL receptors on cell membranes; this inhibits hepatic *de novo* synthesis of cholesterol (Wei *et al*., 2016). Thus, LDL-derived cholesterol meets the cellular requirements for cholesterol and prevents its over accumulation by inhibiting *de novo* cholesterol synthesis, suppressing further entry of LDL, and storing unused cholesterol as cholesteryl esters or exporting it from the liver

as bile acids or other sterol-derived products. About 75 % of high-affinity LDL uptake occurs in the liver. Despite this elaborate regulatory system, cells can accumulate excessive amounts of cholesteryl esters when the plasma LDL saturates the high-affinity, receptor mediated LDL uptake process. Under these conditions, LDL enters cells by a nonspecific endocytic process known as bulk-phase pinocytosis. This mechanism seems to play no role in regulation of *de novo* synthesis of cholesterol and leads to its excessive accumulation, with pathological consequences (e.g., atherosclerosis) (Koekemoer *et al.,* 2017).

High density lipoproteins (HDL) are secreted in nascent form by hepatocytes and enterocytes. Loss of surface components, including phospholipids, free cholesterol, and protein from chylomicrons and VLDL, as they are acted on by lipoprotein lipase, may also contribute to the formation of HDL in plasma. Discoid, nascent HDL is converted to spherical, mature HDL by acquiring free cholesterol from cell membranes or other lipoproteins. HDL eliminates excess cholesterol from peripheral cells and returns it to the liver for excretion. This function of HDL in peripheral cholesterol removal may underlie the strong inverse relationship between plasma HDL levels and the incidence of coronary heart disease (Garcia-Rios *et al.*, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection and identification of seed samples

The seeds of watermelon, fluted pumpkin and moringa were purchased from a market in Madalla, Niger State, Nigeria.

3.2 Methods

The seeds were cleaned of stones, sand and other particles, each of the seeds was divided into four (4) portions and each processed differently.

3.2.1 Methods of processing

3.2.1.1 *Boiling*

The methods described by Ahamefule *et al.* (2008) and Jimoh *et al.* (2014) were used to prepare the boiled samples. The seed sample (1 kg), at 200 g/l of distilled water, was subjected to boiling at 100 °C for 15 min. After which the water was drained off by means of 10 mm sieve and the boiled seeds spread on hessian sacks for 48 to 72 h under sunlight until dry. After drying, the seeds were milled and kept in plastic containers at room temperature for further analysis.

3.2.1.2 *Soaking*

The methods described by Sotolu and Faturoti (2008); Kayembe and Jansen, (2013) and Saulawa *et al.* (2014) were adopted to prepare the soaked samples. One kilogramme (1 kg) of each seed sample was weighed out into 5 L plastic containers, filled with 5 L cold water for 24 h at room temperature. Thereafter, water was drained by means of 10 mm sieve and the soaked seed was spread on hessian sacks for 72 h under sunlight to dry.

After drying, the seeds were milled and kept in plastic containers at room temperature for further analysis.

3.2.1.3 *Germination*

Germination was achieved as described by Kayembe and Jansen, (2013), by soaking the seeds for 24 h. Afterwards they were spread indoors on hessian sacks on the floor, covered with aluminum foil to exclude light, and were allowed to germinate for three days. One litre of water was applied (sprinkle) once daily to provide moisture during sprouting. Thereafter, the germinating seed was dried for 72 h under sunlight, ground and then kept at room temperature pending further analysis.

3.3 Proximate Analysis

Proximate composition of the unprocessed and various processed seed samples were determined at Research Laboratory of the Department of Biochemistry, School of Life Sciences, Federal University of Technology, Minna, Niger State, according to the procedure of AOAC (2006).

3.3.1 Determination of moisture content

The moisture content of the sample was determined according to the method of AOAC (2006). The weight of a dry sterile evaporating crucible was taken after cooling in a desiccator. Two grams of the sample was weigh into preweighed dry evaporating crucible. The crucible and the sample were reweighed before placing them in a hot air oven at a temperature of 105 ^{O}C for 3 h. After drying, the crucible with the sample were allowed to cool in a desiccator containing silica gel. The crucible and the dry samples was reweighed. The procedure of drying was repeated three times until a constant weight is obtained. The moisture content was calculated as a percentage weight loss after drying as follows:

$$\%\ Moisture = \frac{W1-W2}{Weightofsample}\ x\ 100 \qquad (3.1)$$

Where:

W1 = Beginning mass of crucible + Sample 1

W2 = End mass of crucible + Sample 2

3.3.2 Determination of ash content

The ash content analysis was carried out as described by AOAC (2006) method. Two gram (2 g) of sample was weighed into a pre -weighed porcelain crucible and was incinerated in a muffle furnace for 4 h at 600 °C. The furnace was allowed to cool down to approximately 250 °C. The crucibles were removed and placed in a desiccator for 30 min and weigh. The ash content of the sample was calculated as follows:

$$\% \text{ Ash} = \frac{\text{weight of ash}}{\text{sample weight}} \times 100 \qquad (3.2)$$

$$= \frac{W3 - W1}{W2 - W1} \times 100$$

Where W_1= weight of empty crucible

W_2= Weight of crucible + sample before drying and / or ashing

and W_3= Weight of crucible + Ash

3.3.3 Determination of crude fibre

The crude fibre concentration of the sample was determined according to Goering and Van Soest (1970). Two (2) grams of each sample was weighed into a filter crucible and placed on the hot extraction units of the Tecator fibretec system. The extraction was carried with a 30 ml of 98 % sulphuric acid (H_2SO_4) for 14 min, by boiling. The H_2SO_4 was removed by switching on the vacuum pump and washed out (three times) with warm distilled water. Sequential to the H_2SO_4 removal, 100 ml of sodium hydroxide (NaOH) was added and boil for 14 mins before removing it. The residues in the crucibles was dried at 100 °C overnight, then cool in a desiccator for 30 min and weigh.

After weighing, they were ashed in a muffle furnace at 600 °C for 3 h. The furnace was allowed to cool to at least 250 °C, and then the crucibles were cooled in a desiccator for 30 min and weighed.

Percentage CF was calculated as

$$\% CF = \frac{W1\ (g) - W2\ (g)}{W3\ (g)} X\ 100 \qquad (3.3)$$

Where: W1 = dry mass of sample after extraction

W2 = mass of ash

W3 = sample mass

3.3.4 Determination of crude protein

The nitrogen (N) concentration of the sample was determined by the Macro Kjeldahl method (AOAC, 2006). Two grams of the sample was weighed into a digestion flask. A digestion mixture of 10 g sodium sulphate (Na_2SO_4) and 0.4 g elemental selenium was added together with 25 ml of concentrated (98 %) sulphuric acid (H_2SO_4) into the same digestion flask with the sample. The flask was put on a block digester until the solution is clear (45 min) for the digestion of the sample. After cooling of the solution, 35 ml of boric acid solution (40 g solution of H_2BO_3 in 10 mL methyl red and 25 ml methyl blue made up to a 1000 ml) was added. Zinc granules, 350 ml of distilled water and 100 ml NaOH (45 %) were added as well. Then the solution was allowed to boil for about 10 min until about 200 ml of the distillate is remaining for the distillation. The distillate will be titrated with 0.1M HCl. The values were calculated by the titration of a blank sample.

The percentage of N in a sample was calculated as follows:

(3.4)

Where F - factor associated with the strength of the H_2SO_4

Percentage CP will be calculated as follows:

$$\%CP = \%\ \mathrm{N}\ X\ 6.25 \qquad (3.5)$$

$$\%\ \text{Protein} = \%\ \text{Nitrogen} \times 6.25 \qquad (3.5)$$

3.3.5 Determination of crude lipid

Soxhlet method method described by AOAC (2002), was used to determine the crude lipid content of each seed sample. Five (5) grames of each sample was taken into a thimble of known weight (W_1), they together weigh W_2., the thimble with the sample was placed inside a soxhlet extractor 300 ml of petroleum ether was poured into a 500 ml round bottom ground joint flask, which was sited in an electrically connected heating mantle. The heating and extraction process was continued for 24 h, after which the thimble with content was removed dried in an oven at 50 ^{o}C for 24 h, cooled in a dessicator and weighed (W_3). The percentage lipid content of each sample was calculated.

$$\%\ \text{Lipid} = \frac{100(W2-W3)}{W2-W1} \qquad (3.7)$$

W1= weight of thimble

W2= weight of sample and thimble

W3= weight of dried sample and thimble

3.3.6 Carbohydrate content determination

Total carbohydrate content was determined by the method described by Onwuka (2005). The total amount of crude protein, crude fat, moisture and ash of each of the samples was added and subtracted from 100. The percentage carbohydrate was the value obtained.

$$\%\ \text{Carbohydrate} = 100 - (\%\ \text{moisture} + \%\ \text{ash} + \%\ \text{protein} + \%\ \text{fat}) \qquad (3.8)$$

The caloric values of the samples were obtained by multiplying the value of the carbohydrate, crude fat and crude protein by 4, 9, 4 Kcal respectively and taking the sum of the product. i.e. Energy value = (Carbohydrate x 4) + (Fat x 9) +(Protein x 4).

3.4 Mineral Content Digestion

The minerals were digested using dry ashing method as described by Nielsen (2002). Two (2) grams of the samples were ashed in a furnace at 550 °C for 4 h. The ash was removed from the furnace after ashing and kept for it to cool. The ash was leached with 5 ml of 6 M HCl. The volume was made up to 20 cm^3 with distilled water. The blank determination was also carried out in a similar way as described above except for the omission of sample. Potassium in the solution was determined by flame photometry method using an instrument called Flame Emission Spectrophotometer. Phosphorus was determined by Vanadomolybdate method and absorption was read using spectrophotometer, while other selected minerals (calcium, magnesium and iron) were determined from the resulting solution using Atomic Absorption Spectrophotometer (AAS Model SP9).

3.5 Amino acid Analysis

The Amino acid profile of the seeds sample was determined using methods described by Benitez (1989). The dried sample was defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample (TSM) Amino acid Analyser

3.5.1 Defatting method

The sample was defatted using chloroform/methanol mixture (ratio 2:1). Four grams (4g) of the sample was put in extraction thimble and Fat was extracted for 15 h in soxhlet extraction apparatus (AOAC, 2006). The nitrogen content of defatted sample was determined using Kjedahl method (AOAC, 2006).

3.5.2 Hydrolysis of the sample

Two grams (2 g) of the defatted seed sample was weighed into glass ampoule. Seven milliliter (7 ml) of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids like methionine and cystine during hydrolysis). The glass ampoule was sealed with bunsen burner flame and put in an oven preset at 105 ^{O}C ± 5 ^{O}C for 22 h. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness in hot air oven. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottle for further analysis (AOAC, 2006).

3.5.3 Hydrolysis of sample for tryptophan determination

Two grams (2 g) of the defatted sample was weighed into glass ampoule. 10 ml of 4.2 M NaOH was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 ^{O}C ± 5 ^{O}C for 22 h. The ampoule was allowed to cool before it was broken at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40 ^{O}C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 8.0) and stored in plastic specimen bottle for further analysis.

3.5.4 Sample loading

Five microliter (5 µl) of sample was dispensed into the cartridge of the analyser. The eTechnicon sequential Multi-Sample (TSM) analyser is designed to separate and analysed free acidic, neutral and basic amino acids of the hydrolysate.

3.5.5 Method of calculating amino acid values from chromatogram peaks

The net height of each peak produced by the chart recorder of Technicon sequential Multi-Sample (TSM) (each representing an amino acid) was measured. Area of each peak was then obtained by multiplying the height with the width at half-height. The nor leucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

$$NE = \frac{\text{Area of Norleucine Peak}}{\text{Area of each amino acid}} \qquad (3.9)$$

A constant S was calculated for each amino acid in the standard mixture:

Where $S_{std} = NE_{std}$ x Molecular weight x µ MAA_{std}

Finally, the amount of each amino acid present in the sample was calculated in g /16 g N or g /100 g protein using the following formula:

Concentration (g /100 g protein) = NH x W at NH/ 2 x S_{std} x C

Where $C = \frac{\text{Dilution} \times 16}{\text{SampleWt(g)} \times N\% \times 10 \times \text{Vol loaded}} \div NH \times W \text{ (nleu)}$

Where: NH = Net height

W = Width at half height

nleu = Norleucine

3.6 Vitamin Analysis

The vitamin E, C, K, B_2 and B_{12} contents of the seed samples were carried out using various standard analytical procedures.

3.6.1 Vitamin E determination

Vitamin E content was determined by the method described in the (Marck, 2001). One gram (1 g) of seed sample was weighed and 10 mL of methanol was added. The sample was homogenised and then filtered. 0.4 mL of the extract was taken and 7.6 ml of colour developer (containing sodium dihydrogen phosphate (0.84 g), ammonium molybdate (1.24 g), H_2SO_4 (8.15 ml) and 250 ml methanol) were added to the sample. 0.4 mL of methanol was then added to the sample. The sample was incubated at 90 ^{0}C for 1 h. The absorbance of the sample was read at 695 nm using spectrophotometer. Concentration of vitamin E was extrapolated from standard curve that was prepared.

3.6.2 Vitamin B_{12} determination

Vitamin B_{12} content was determined by the method described in the (Marck, 2001). One gram (1 g) of seed sample was weighed into a 250 ml volumetric flask. 100 ml of distilled water was added to the flask which was shaken for 45 min and made up to mark with distilled water. The sample was then filtered into a 250 ml beaker. To 20 ml of the filtrate and 5 ml of 1 % sodium dithionite solution were added to decolorize the yellow colour. Standard cyanocobalamin with concentrations ranging from 0 – 10 µg/ml was prepared from stock cyanocobalamin and used to obtain a gradient factor. The absorbance of sample as well as standard was read at a wavelength of 445 nm on a spectrophotometer.

$$\text{Vitamin } B_{12}\ (\mu g)\ /100\ g = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{weight of sample}} \qquad (3.10)$$

3.6.3 Vitamin K determination

Vitamin K content was determined using the method described by Rohde *et al*. (2007). Five gram (5 g) of seed sample was weighed in a 250 ml beaker and 30 ml of butyl alcohol was added. The mixture was thoroughly shaken to obtain a homogenous

solution. The resulting mixture was filtered through a filter paper into a volumetric flask and made up to mark with butyl alcohol. 10 ml of the sample was pipetted into a 30 ml centrifuge tube and 3 drops of 2, 4 – dinitophenyl hydrazine was added to develop a blue colour which changed subsequently to a bluish green colour on addition of mL alcoholic ammonia. Standard solutions of vitamin K from 0 – 20 µg/ml was prepared to obtain a gradient factor. The absorbance of standards and sample was read on a spectrophotometer at a wavelength of 480 nm.

$$\text{Vitamin K in µg /100 g} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{weight of sample}} \qquad (3.11)$$

3.6.4 Vitamin B_2 determination

The determination of vitamin B_2 was carried out based on the method described by Rohde *et al.* (2007). Two grams (2 g) of seed sample was weighed, crushed and dissolved in 20 ml of glycerinated phosphate buffer. This was centrifuged for 10 min. The supernatant of the sample was obtained and 10 ml of the sample was taken into a 100 ml volumetric flask and made up to the mark with distilled water. 10 ml of both test and standard solutions (Riboflavin) were pippetted into separate 50 ml volumetric flasks and 2 ml of 2 % citric acid solution and $KMnO_4$ were added to the sample and allowed to stand for 2 min. Finally 1 ml of H_2O_2 was added to both flask containing the test and standard solutions and solutions was allowed to stand for 5 min. The absorbance of the test and standard solutions was taken at 450 nm. Vitamin B2 content was calculated using the formula

$$\text{Vitamin B2 in mg /2 g} = \frac{AT}{AS} - 0.085 \times \frac{WS}{500} \times 2 \qquad (3.12)$$

Where AT is absorbance of Test

AS is absorbance of standard sample

WS is weight of standard sample

3.6.5 Vitamin C determination

Vitamin C content was determined using the method described by Onwuka (2005). Five grams (5 g) of the sample was homogenised in 45 ml of distilled water. The suspension was then filtered. 5 mL of the filtrate was measured into a 250 ml conical flask and 0.1 ml of glacial acetic acid was added. Dichlorophenol indophenol was titrated against the filtrate in the flask until the solution became faint pink. The titre value was taken and the vitamin C content was then calculated

3.7 Determination of Antinutrients

The antinutrients to be determined in the seed samples were cyanogenic glycoside, phytate, tannins, oxalates and saponins.

3.7.1 Determination of cyanogenic glycosides

Alkaline picrate method as described by Onwuka (2005) was used to determine the cyanogenic glycoside content of the seed flour. Five (5 g) of seed flour was dissolved in 50 ml distilled water in a conical flask and allowed to stay overnight to extract cyanide. The extract was filtered and the filtrate used for cyanide determination. To 1 ml of sample filtrate, 4 ml of alkaline picrate was added and allowed to incubate in a water bath for 5 min. After colour development (reddish brown colour), the absorbance was read at 490 nm. The cyanide content was extrapolated from a cyanide standard curve.

3.7.2 Determination of phytates

Phytate was determined using the method of AOAC (2000). Ten grams (10 g) of seed flour was weighed into the conical flasks and then extracted with 50 ml of 3 % TCA for 45 min with occasional swirling by hand. The phytate was precipitated as ferric phytate with the solution of iron (III) chloride. The precipitate was converted to sodium phytate with 3 % solution of NAOH before digesting with an acid mixture of equal portion of

concentrated H_2SO_4 and 65 % ClO_4. The liberated phosphorus was quantified calorimetrically at 620 nm after colour development with ammonium molybdate to which sodium sulphate and hydroquinone solutions was added.

Concentration of phytate phosphorus = $\frac{Titre\ value\ x\ 0.601\ phytate}{1000\ x\ weitght\ of\ sample}$ (3.13)

3.7.3 Determination of tannins

The Folin Denis Spectrophotometric method was employed as described by Onwuka (2005) to determine the tannin content of seed flour. One gram (1 g) of each seed flour was dispersed in 10 ml distilled water and shaken. The mixture was allowed to stand for 30 min at room temperature. At the end of 30 min, the mixture was centrifuged and the extract obtained. 2.5 ml of the supernatant (extract) was transferred into a 50 mL volumetric flask. Similarly 2.5 ml of standard tannic acid solution was transferred into a separate 50 ml flask. 1 ml of Folin – Denis reagent was measured into each flask, followed by 2.5 ml of saturated Na_2CO_3 solution. The mixture was diluted to mark in the flask (50 ml) and incubated for 90 minutes at room temperature. The absorbance was measured at 250 nm using Spectrophotometer. The tannin content was calculated as follows:

% Tannin = $\frac{An \times C \times 100 \times Vf}{As \times W}$ (3.14)

Where An = absorbance of test sample, As = absorbance of standard solution. C= concentration of standard solution, W = weight of sample, Vf = total volume of extract.

3.7.4 Determination of oxalates

The oxalate content of seed samples was determined using the method described by AOAC (2000). Two gramme (2 g) of the seed flour was weighed into a 250 ml beaker; 190 ml of distilled water and 10 ml of 6 molar HCl was added to the beaker. It was allowed to stand for 5 min while mixing it at intervals of 30 sec. The volume was made

up to 250 ml with distilled water after which 50 ml was measured out and titrated using few drops of methyl red indicator while adding drop by drop concentrated ammonium until the colour become faint yellow. It will then be heated on steam water bath to boil, removed and allowed to cool before filtering and heating again to boil. 10 ml of 5 % $CaCl_2$ was added while constantly stirring and another 5 ml of $CaCl_2$ was added to give more precipitate of oxalate from the sample. This was removed and allowed to stand overnight after which it was filtered, the precipitate was washed into a beaker using 1:4 H_2SO_4 acids and then rinsed with 5 ml of hot distilled water. The solution was heated and titrated against 0.05 N $KMnO_4$. The titre value of the blank was subtracted from that of the sample and multiplied by 50 to get the result in mg /100 g of the sample.

Calculation: 1 m of 0.05 N $KMnO_4$ = 3.0 mg Oxalate.

3.7.5 Determination of saponins

The standard method of AOAC (2000) was used to determine saponins in the seed flour. 1 g finely ground seed flour was weighed into a 250 ml beaker and 100 ml of methyl alcohol was added. The mixture was shaken on a shaker for 5 h to ensure uniform mixing. Thereafter, the mixture was filtered through filter paper into a 100 ml beaker and 20 ml of 40 % saturated solution of magnesium carbonate was added. The mixture obtained with saturated $MgCO_3$ will again be filtered through a filter paper to obtain a clear colourless solution. 1 ml of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5 % $FeCl_3$ solution was added and make up to mark with distilled water. The reaction was stopped for 30 min to allow blood red colour to develop. 0 -10 ppm standard saponnin was prepared from saponnin stock solution. The standard solution was treated similarly with 2 ml of 5 % $FeCl_3$ solution as done for 1 ml sample above. The absorbance of the sample as well as standard saponnin solutions was read after colour development on a spectrophotometer at a wave length of 380 nm.

$$\%\ Saponins = \frac{Absorbance\ of\ sample\ x\ Gradient\ factor\ X\ dilution\ factor}{Weight\ of\ sample}\ X\ 100 \quad (3.15)$$

3.8 Determination of Functional Properties of Seeds Flour

The functional properties of flours that was analyzed are, swelling capacity (mL), water absorption capacity (WAC, %), oil absorption capacity (OAC, %), emulsion activity (EA, %), emulsion stability (ES, %), foam capacity (FC, %), foam stability (FS, %), gelatinization temperature (GT, °C), least gelatinization concentration (LGC, %) and bulk density (g/ml).

3.8.1 Swelling capacity

The swelling capacity was determined by the method described by Okaka and Potter (1977) where 100 mL graduated cylinder was fill with the sample to 10 mL mark. Distilled water was added to give a total volume of 50 mL. The top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. The suspension was inverted again after 2 minutes and was left to stand for a further 8 min and the volume occupied by the sample was taken after the eighth minutes.

3.8.2 Water absorption capacity

The water absorption capacity of the flours was determined by the method of Sosulski *et al*. (1976). One gram of sample was mixed with 10 mL distilled water and allowed to stand at ambient temperature (30 ± 2 °C) for 30 min, the centrifuged for 30 minutes at 3000 rpm or 2000 g.

3.8.3 Oil absorption capacity

Oil absorption was examined as percent oil bound per gram flour. The oil absorption capacity was determined using the method described by Sosulski *et al.* (1976) method. One gram (1 g) of sample was mixed with 10 ml soybean oil (Sp. Gravity 0.9092) and

allowed to stand at ambient temperature (30 ± 2 °C) for 30 min, then centrifuged for 30 min at 300 rpm or 2000 g. Water absorption was examined as percent water bound per gram flour.

3.8.4 Emulsion activity and stability

The emulsion activity and stability were determined using the method decribed by Yasumatsu *et al.* (1972). For emulsion (1 g sample, 10 ml distilled water and 10 ml soybean oil) was prepared in calibrated centrifuged tube. The emulsion was centrifuged at 2000 g for 5 min. The ratio of the height of emulsion layer to the total height of the mixture was calculated as emulsion activity in percentage. The emulsion stability was estimated after heating the emulsion contained in calibrated centrifuged tube at 80 °C for 30 min in a waterbath, cooling for 15 min under running tap water and centrifuging at 2000 g for 15 min. The emulsion stability expressed as percentage was calculated as the ratio of the height of emulsified layer to the total height of the mixture.

$$Emulsifying\ activity = \frac{Height\ of\ emulsified\ layer}{Height\ of\ total\ contents\ in\ the\ tube}\ X\ 100 \qquad (3.16)$$

3.8.5 Foam capacity and stability

The foam capacity (FC) was determined as described by Narayana and Narasinga (1982) was determined as described with slight modification. The 1.0 g flour sample was added to 50 ml distilled water at 30 ± 2 °C in a graduated cylinder. The suspension was mixed and shaken for 5 min to foam. The volume of foam at 30 sec after whipping willbe expressed as foam capacity using the formula:

$$\text{foam capacity (\%)} = \frac{\text{volume of foam (AW)} - \text{volume of foam (BW)}}{\text{volume of foam (BW)}}\ \text{x } 100 \qquad (3.17)$$

Where, AW = after whipping, BW = before whipping.

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The volume of foam was recorded one hour after whipping to determine foam stability as per percent of initial foam volume.

3.8.6 Apparent (bulk) density

The (bulk) density was determined using the method of Jones *et al*. (2000), which says volume of 100 g of the flour was measured in a measuring cylinder (250 ml) after tapping the cylinder on a wooden plank until no visible decrease in volume was noticed, and based on the weight and volume, the apparent (bulk) density was calculated.

3.9 Animal Studies

Seventy-eight weanling albino rats weighing 70 g to 100 g were used in this study. The animals were acclimatized for the period of one week prior to the start of the experiment. They were allowed to housed in Biochemistry Animal House, Federal University of Technology, Minna and as well receive normal rat chows and water *ad libitum*. After a week, they were divided into thirteen groups of six animals each. The experimental diet and water was offered *ad libitum* to the rats for 28 days. The rats were weighed to obtain their initial weights so that their weight changes can be determined at the end of experimental feeding.

The experimental diet was formulated based on the American institute of nutrition (AIN) method as describe by Reeves *et al. (*1993*)*.

Table 3.1: Composition of *Telfaira occidentalis* (Fluted pumpkin) Seed Flours based diet (per kg diet)

Ingredients	Control Diet	Diet Containing unprocessed Seed (g)	Diet Containing boiled Seed (g)	Diet Containing soaked Seed (g)	Diet Containing germinated Seed (g)
Seed Sample	-	400	350	440	300
G.nut cake	130	-	-	-	-
Cornflour	670	400	450	360	500
G.nut oil	5	5	5	5	5
Maize bran	100	100	100	100	100
Bone meal	25	25	25	25	25
Glucose	43	43	43	43	43
Premix	5	5	5	5	5
Salt	17	17	17	17	17
Methionine	3	3	3		3
Lysine	2	2	2	2	2

Premix Composition (1.25 kg)

Vitamin D3 (1,7000,000 IU), Vitamin A (8,000,000 IU), Vitamin E (5,000 mg), Vitamin K3 (1,500 mg), Folic acid (200 mg) Niacin (15,000 mg), Vitamin B2 (3,000 mg), Vitamin B12 (5 mg), Vitamin B1(1000 mg), Vitamin B6 (1000 mg), Biotin, Antioxidant (125,000 mg) Calpan (5,000 mg).Cobalt (100 mg), Selenium (100mg), Iodine (1,000 mg), Iron 25,000 mg, Manganese (45,000 mg), Copper (3,000 mg), Zinc (35,000 mg), Choline Chloride (100,000 mg)

Source: Abdel-Shafy *et al.*, (2011)

Table 3.2: Composition of *Moringa oleifera* (Moringa) Seed Flours based diet (per kg diet)

Ingredients	Control Diet	Diet Containing unprocessed Seed (g)	Diet Containing boiled Seed (g)	Diet Containing soaked Seed (g)	Diet Containing germinated Seed (g)
Seed Sample	-	260	380	270	210
G.nut cake	130	-	-	-	-
Cornflour	670	540	420	530	590
G.nut oil	5	5	5	5	5
Maize bran	100	100	100	100	100
Bone meal	25	25	25	25	25
Glucose	43	43	43	43	43
Premix	5	5	5	5	5
Salt	17	17	17	17	17
Methionine	3		3		3
Lysine	2	2	2	2	2

Premix Composition (1.25 kg)

Vitamin D3 (1,7000,000 IU), Vitamin A (8,000,000 IU), Vitamin E (5,000 mg), Vitamin K3 (1,500 mg), Folic acid (200 mg) Niacin (15,000 mg), Vitamin B2 (3,000 mg), Vitamin B12 (5 mg), Vitamin B1(1000 mg), Vitamin B6 (1000 mg), Biotin, Antioxidant (125,000 mg) Calpan (5,000 mg).Cobalt (100 mg), Selenium (100mg), Iodine (1,000 mg), Iron 25,000 mg, Manganese (45,000 mg), Copper (3,000 mg), Zinc (35,000 mg), Choline Chloride (100,000 mg)

Source: Abdel-Shafy *et al.*, (2011)

Table 3.3: Composition of *Citrullus lanatus* (Watermelon) Seed Flours based diet (per kg diet)

Ingredients	Control Diet	Diet Containing unprocessed Seed (g)	Diet Containing boiled Seed (g)	Diet Containing soaked Seed (g)	Diet Containing germinated Seed (g)
Seed Sample	-	680	490	610	620
G.nut cake	130	-	-	-	-
Cornflour	670	120	310	190	180
G.nut oil	5	5	5	5	5
Maize bran	100	100	100	100	100
Bone meal	25	25	25	25	25
Glucose	43	43	43	43	43
Premix	5	5	5	5	5
Salt	17	17	17	17	17
Methionine	3	3	3	3	3
Lysine	2	2	2	2	2

Premix Composition (1.25 kg)

Vitamin D3 (1,7000,000 IU), Vitamin A (8,000,000 IU), Vitamin E (5,000 mg), Vitamin K3 (1,500 mg), Folic acid (200 mg) Niacin (15,000 mg), Vitamin B2 (3,000 mg), Vitamin B12 (5 mg), Vitamin B1(1000 mg), Vitamin B6 (1000 mg), Biotin, Antioxidant (125,000 mg) Calpan (5,000 mg).Cobalt (100 mg), Selenium (100mg), Iodine (1,000 mg), Iron 25,000 mg, Manganese (45,000 mg), Copper (3,000 mg), Zinc (35,000 mg), Choline Chloride (100,000 mg)

Source: Abdel-Shafy *et al.*, (2011)

Table 3.4: Experimental Animal Design

Group	Diet
1	Control
2	Unprocessed Watermelon Seed Flour Based Diets
3	Boiled Watermelon Seed Flour Based Diets
4	Germinated Watermelon Seed Flour Based Diets
5	Soaked Watermelon Seed Flour Based Diets
6	Unprocessed Moringa Seed Flour Based Diets
7	Boiled Moringa Seed Flour Based Diets
8	Germinated Moringa Seed Flour Based Diets
9	Soaked Moringa Seed Flour Based Diets
10	Unprocessed Fluted Pumpkin Seed Flour Based Diets
11	Boiled Fluted Pumpkin Seed Flour Based Diets
12	Germinated Fluted Pumpkin Seed Flour Based Diets
13	Soaked Fluted Pumpkin Seed Flour Based Diets

3.10 Collection of Blood Samples

At the end of the 28 days feeding experiment, the rats were weighed to obtain their final weights. Each rat was anaesthetized under diethylether in a desiccator and euthanized. Blood samples were collected in EDTA sample bottles for haematological analysis and plain bottles for biochemical analysis. Blood samples collected for biochemical parameters were spinned using bench-top centrifuge at 3000 rpm for 10 min to separate the serum from the plasma.

3.11 Assays for Haematological Parameter

Blood samples were collected into sample bottles containing a speck of tetra acetic ethylene diamine acid (EDTA) powder. The following haematological parameters were determined, packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (Hb) and mean corpuscular haemoglobin concentration (MCHC) were determined by using automated hematology analyzer following the manufactures instruction (Mindray 3000 plus).

3.12 Determination of Lipid Profile

Serum cholesterol level was measured using the method described by Allain *et al.* (1974). High density lipoprotein content was determined as described by Albers *et al.* (1978). Low density lipoprotein content was determined using the method of Assman *et al.* (1984). Triglyceride was determined using glycerol-phosphate oxidase method described by Albers *et al.* (1978).

3.13 Determination of Serum Biochemical Analysis

The aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity were determined by Reitman and Frankel (1957). Plasma creatinine and plasma electrolytes were measured within 24 hours. At the end of the experiment using methd decribed by Silbernagl (1986).

3.14 Isolation of Organs from Rats for Histopathology Investigation

The histopathology analysis of the liver and kidney excised from the experimental animals were carried out in the histopathology laboratory of the University of Abuja Teaching Hospital, Gwagwalada Abuja, Nigeria. The various organs were processed using Automatic tissue processor Model SLEE MTP. The tissues were fixed with 10 % neutral buffered formalin for 1 h and afterwards dehydrated with ethanol (70, 95 and 100 %) at different concentration in ascending concentration and different time in order to remove water from the tissues. Thereafter, clearing with xylene was done for 2 h to remove alcohol and prepare the tissue for waxing. Impregnation of the tissue was then carried out using moulting paraffin wax to remove xylene. Embedding was done using embedding machine (Raymond A Lamb Blockmaster II) by burying the tissue in paraffin wax using embedding mould and tissue cassettes. Sectioning of the tissue was carried out using a Rotary microtome machine MRS 3500 and the tissue was cut at a thickness of 5 microns. The section was floated on a water bath (Light water, Surrey GU 185TA, UK), maintained at a temperature 2 ^{O}C below the melting point of the wax to pick the section unto the slide. The slide was then placed on a hot plate (Biotec), maintained at a temperature 2 ^{O}C above the melting point of the waxto enable the tissue to stick to the slide. The slide was dewaxed using xylene for 2 min and treated with alcohol (100 %, 95 % and 70 %). Thereafter, staining was done with Harris hematoxylin for 5 min to stain the nucleus while eosin was used to stain the cytoplasm for 3 min. Dehydration was again carried out in alcohol and alcohol cleared with xylene. A mounting medium, dibutylphthalate xylene (DPX) was dropped on the tissue section, which were viewed through the microscope (Olympus CX21) (Saberi *et al.*, 2017).

3.15 Data Analysis

Statistical analyses was performed using analysis of variance (ANOVA) followed by Duncan's multiple range tests using SPSS program 20.0. p values ≤ 0.05 was considered to be significant and all values were expressed as mean ± SEM.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Nutritional composition of unprocessed and processed flours from watermelon, moringa and fluted pumpkin seeds

This revealed the results of the proximate composition, mineral content, vitamin content and amino acid profile of unprocessed and processed *Citrullus lanatus*, *Moringa oleifera* and *Telfaira occidentalis* seed flours.

4.1.1.1 *Proximate composition of unprocessed and processed Citrullus lanatus, Moringa oleifera and Telfaira occidentalis seed flours*

The proximate composition of unprocessed and processed flours from watermelon, moringa and fluted pumpkin seeds are reported below. Table 4.1a, 4.1b and 4.1c shows the proximate composition of water melon, moringaand fluted pumpkin seed flours respectively.

The proximate composition of both unprocessed and processed watermelon seed flour is shown in Table 4.1a. There was a significant increase ($p < 0.05$) in the crude protein and fat contents, and energy values of the boiled (18.77 %) and soaked seed (16.90 %) flours of watermelon when compared to the its unprocessed seed flour (14.00 %), significant decrease ($p < 0.05$) was observed in moisture content and fibre of all the processed seed flour of watermelon when compare to the unprocessed seed flour. Significant increased ($p < 0.05$) was seen in the ash content of all the processed seed flour of watermelon and in carbohydrate of germinated seed flour of watermelon when compared to the unprocessed watermelon seed flour.

The proximate composition of both unprocessed and processed moringa seed flour is revealed in Table 4.1b. Significant increase ($p < 0.05$) was seen in the crude protein

content of germinated seed flour of moringa. Processing significantly increased the fat content of moringa flours.

Proximate composition of both unprocessed and processed fluted pumpkin seed flour is been laid out in Table 4.1c. There was significant increase ($p < 0.05$) in the crude protein content of germinated (25.70 %) and boiled (22.20 %) seed flours of fluted pumpkin when compared to its unprocessed (21.07 %) .On a general note, significant difference ($p < 0.05$) was observed in the carbohydrate, protein, fibre, ash, moisture and fat contents of all the processedseed flour when compared to their respective unprocessed seed flour. There was a significant difference ($p < 0.05$) in the metabolized energy of all the seeds at different stages.

Table 4.1a: Proximate Composition of Unprocessed and Processed *Citrullus lanatus* (Watermelon) Seed Flour

Proximate composition (%)	UWMSF	SWMSF	GWMSF	BWMSF
Moisture	3.56 ± 0.03^{c}	0.04 ± 0.28^{a}	1.26 ± 0.01^{b}	0.31 ± 0.02^{a}
Ash	1.70 ± 0.15^{a}	3.83 ± 0.09^{b}	4.45 ± 0.02^{c}	4.93 ± 0.23^{d}
Carbohydrate	33.20 ± 1.11^{a}	40.41 ± 0.77^{d}	38.87 ± 1.55^{c}	36.92 ± 0.82^{b}
Protein	14.00 ± 1.01^{a}	16.90 ± 0.60^{b}	13.25 ± 1.70^{a}	18.77 ± 0.63^{c}
Fibre	21.09 ± 0.67^{d}	9.40 ± 0.58^{a}	19.36 ± 0.21^{c}	12.25 ± 0.25^{b}
Fat	26.45 ± 0.03^{b}	29.05 ± 0.07^{c}	22.64 ± 0.03^{a}	26.82 ± 0.02^{b}
Energy (Kcal)	426.85 ± 0.83^{b}	490.69 ± 1.04^{d}	412.24 ± 0.22^{a}	464.14 ± 0.96^{c}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SWMSF –Soaked Watermelon Seed Flour
GWMSF- Germinated Watermelon Seed Flour
BWMSF- Boiled Watermelon Seed Flour
UWMSF-Unprocessed Watermelon Seed Flour

Table 4.1b: Proximate Composition of Unprocessed and Processed *Moringa oleifera* (Moringa) Seed Flour

Proximate composition (%)	UMSF	SMSF	GMSF	BMSF
Moisture	1.11 ± 0.02^{c}	0.21 ± 0.02^{b}	0.23 ± 0.01^{b}	0.04 ± 0.01^{a}
Ash	4.23 ± 0.09^{d}	3.47 ± 0.04^{c}	3.18 ± 0.01^{b}	3.00 ± 0.06^{a}
Carbohydrate	28.24 ± 0.64^{b}	29.83 ± 2.51^{b}	24.07 ± 2.37^{a}	35.61 ± 2.20^{c}
Protein	27.43 ± 0.57^{b}	26.87 ± 2.53^{b}	32.61 ± 0.05^{c}	21.60 ± 2.11^{a}
Fibre	13.61 ± 0.20^{b}	13.16 ± 0.12^{a}	13.61 ± 0.05^{b}	13.23 ± 0.04^{a}
Fat	25.38 ± 0.01^{a}	26.48 ± 0.02^{b}	26.78 ± 0.02^{c}	26.82 ± 0.02^{d}
Energy (Kcal)	451.10 ± 0.83^{a}	465.12 ± 0.94^{b}	467.74 ± 0.52^{a}	470.22 ± 0.96^{c}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p< 0.05$)
SMSF- Soaked Moringa seed flour
GMSF- Germinated Moringa seed flour
BMSF- Boiled Moringa seed flour
UMSF-Unprocessed Moringa seed flour

Table 4.1c: Proximate Composition of Unprocessed and Processed *Telfaira occidentalis* (Fluted pumpkin) Seed Flour

Proximate composition (%)	UFSF	SFSF	GFSF	BFSF
Moisture	1.44 ± 0.03^{d}	0.62 ± 0.01^{b}	1.32 ± 0.03^{c}	0.45 ± 0.03^{a}
Ash	4.87 ± 0.09^{c}	2.57 ± 0.15^{a}	4.21 ± 0.05^{b}	4.30 ± 0.17^{b}
Carbohydrate	19.27 ± 3.59^{bc}	17.88 ± 1.41^{c}	14.81 ± 0.70^{a}	16.16 ± 0.70^{b}
Protein	21.07 ± 3.63^{a}	19.90 ± 1.55^{a}	25.70 ± 0.60^{c}	22.20 ± 0.60^{b}
Fibre	0.15 ± 0.05^{a}	2.51 ± 0.09^{c}	2.30 ± 0.03^{b}	2.16 ± 0.05^{b}
Fat	53.20 ± 0.01^{b}	56.52 ± 0.01^{d}	51.73 ± 0.05^{a}	54.73 ± 0.03^{c}
Energy (Kcal)	640.16 ± 0.51^{b}	659.8 ± 0.67^{d}	627.61 ± 0.07^{a}	646.01 ± 0.72^{c}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SFSF- Fluted Pumpkin Soaked Fluted Pumpkin Seed Flour
GFSF- Germinated Fluted Pumpkin Seed Flour BFSF-
Boiled Fluted Pumpkin Seed Flour
UFSF- Unprocessed Fluted Pumpkin Seed Flour

4.1.1.2 *Mineral content of unprocessed and processed Citrullus lanatus, Moringa oleifera Lam. and Telfaira occidentalis seed flours*

The mineral content available in both unprocessed and processed watermelon, moringa and fluted pumpkin seed flours are shown in Table 4.2a, 4.2b and 4.2c respectively. Germination and boiling significantly increased ($p<0.05$) the calcium content of watermelon seed flour as seen in Table 4.2a while all methods of processing significantly increased ($p<0.05$) the potassium and sodium contents of watermelon seed flour. Soaked moringa seed flour was significantly higher in calcium and zinc content when compared to its unprocessed seed flour as revealed in Table 4.2b. Potassium and sodium contents was significantly higher ($p<0.05$) in the unprocessed seed flour of moringa when compared to the processed seed flours.

Calcium was significantly higher ($p<0.05$) in soaked fluted pumpkin flour when compared to its unprocessed seed flour as shown in Table 4.2c, no significant difference ($p>0.05$) in the iron content of germinated and boiled seed flours of fluted pumpkin when compared with their unprocessed.

Generally, the result showed that Ca, Mg, P, K, Na, Mn, Cr, Fe and Zn are present in all the seed flours. The most abundant mineral in all the seeds was phosphorus while manganese was the least abundant mineral in the seed flours.

Table 4.2a: Mineral Composition of Unprocessed and Processed Citrullus *lanatus* (Watermelon) Seed Flour

Mineral Composition (mg/100g)	UWMSF	SWMSF	GWMSF	BWMSF
Ca	1.07 ± 0.00^{b}	0.96 ± 0.00^{a}	1.14 ± 0.01^{c}	1.10 ± 0.00^{c}
Mg	1.07 ± 0.02^{b}	1.09 ± 0.01^{c}	1.08 ± 0.01^{b}	1.04 ± 0.03^{a}
P	40.00 ± 1.15^{b}	36.00 ± 0.58^{a}	38.00 ± 1.15^{a}	45.00 ± 2.89^{c}
K	0.87 ± 0.00^{a}	1.01 ± 0.00^{c}	1.01 ± 0.00^{c}	0.89 ± 0.00^{b}
Na	1.29 ± 0.00^{d}	1.07 ± 0.00^{b}	1.18 ± 0.06^{c}	1.00 ± 0.00^{a}
Mn	0.74 ± 0.01^{b}	0.70 ± 0.06^{a}	0.73 ± 0.01^{a}	0.75 ± 0.01^{b}
Cr	0.77 ± 0.01^{a}	0.76 ± 0.06^{a}	0.77 ± 0.01^{a}	0.80 ± 0.03^{b}
Fe	0.74 ± 0.01^{a}	0.76 ± 0.02^{b}	0.76 ± 0.01^{b}	0.74 ± 0.01^{a}
Zn	12.30 ± 0.02^{c}	10.06 ± 0.01^{a}	11.73 ± 0.09^{b}	12.51 ± 0.01^{c}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SWMSF –Soaked Watermelon Seed Flour
GWMSF- Germinated Watermelon Seed Flour
BWMSF- Boiled Watermelon Seed Flour
UWMSF- Unprocessed Watermelon Seed Flour

Table 4.2b: Mineral Composition of Unprocessed and Processed Moringa *oleifera* (Moringa) Seed Flour

Mineral Composition (mg/100g)	UMSF	SMSF	GMSF	BMSF
Ca	1.18±0.00^{b}	1.21±0.00^{c}	1.10±0.00^{a}	1.10±0.00^{a}
Mg	1.08±0.00^{c}	1.06±0.00^{b}	1.02±0.00^{a}	1.08±0.00^{c}
P	43.00±0.58^{d}	38.00±1.15^{b}	33.00±0.58^{a}	41.00±0.58^{c}
K	1.01±0.00^{d}	0.81±0.00^{a}	0.84±0.00^{b}	0.98±0.00^{c}
Na	1.40±0.00^{b}	1.01±0.00^{a}	1.00±0.00^{a}	1.01±0.00^{a}
Mn	0.71±0.00^{a}	0.74±0.00^{b}	0.75±0.00^{c}	0.71±0.00^{a}
Cr	0.76±0.01^{b}	0.76±0.00^{b}	0.75±0.00^{a}	0.77±0.01^{c}
Fe	0.76±0.04^{b}	0.74±0.02^{a}	0.74±0.01^{a}	0.76±0.03^{b}
Zn	10.08±0.01^{a}	12.7±0.04^{c}	12.46±0.05^{c}	11.21±0.03^{b}

Values are expressed as means of triplicate determinations ± SEM.Values along rows with different superscript are significantly different ($p< 0.05$)
SMSF- Soaked Moringa seed flour
GMSF- Germinated Moringa seed flour
BMSF- Boiled Moringa seed flour
UMSF-Unprocessed Moringa seed flour

Table 4.2c: Mineral Composition of Unprocessed and Processed *Telfaira occidentalis* (Fluted pumpkin) Seed Flour

Mineral Composition (mg/100g)	UFSF	SFSF	GFSF	BFSF
Ca	1.16 ± 0.00^{b}	1.18 ± 0.00^{c}	1.16 ± 0.00^{b}	1.10 ± 0.00^{a}
Mg	1.08 ± 0.00^{b}	1.07 ± 0.04^{a}	1.08 ± 0.00^{b}	1.08 ± 0.01^{b}
P	27.00 ± 1.15^{a}	26.00 ± 1.73^{a}	30.00 ± 2.89^{b}	32.00 ± 1.15^{b}
K	1.81 ± 0.01^{a}	0.85 ± 0.00^{b}	0.86 ± 0.01^{b}	0.99 ± 0.00^{c}
Na	1.92 ± 0.00^{d}	1.01 ± 0.00^{a}	1.16 ± 0.00^{b}	1.55 ± 0.00^{c}
Mn	0.73 ± 0.00^{a}	0.74 ± 0.02^{b}	0.73 ± 0.00^{a}	0.72 ± 0.01^{a}
Cr	0.77 ± 0.01^{b}	0.75 ± 0.00^{a}	0.77 ± 0.00^{b}	0.77 ± 0.00^{b}
Fe	0.76 ± 0.03^{b}	0.74 ± 0.01^{a}	0.76 ± 0.02^{b}	0.76 ± 0.03^{b}
Zn	12.23 ± 0.05^{b}	12.42 ± 0.01^{b}	11.82 ± 0.02^{a}	11.15 ± 0.03^{a}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SFSF- Fluted Pumpkin Soaked Fluted Pumpkin Seed Flour
GFSF- Germinated Fluted Pumpkin Seed Flour BFSF-
Boiled Fluted Pumpkin Seed Flour UFSF- Unprocessed
Fluted Pumpkin Seed Flour

4.1.1.3: *Vitamin Content of unprocessed and processed Citrullus lanatus, Moringa oleifera and Telfaira occidentalis*

The vitamin content available in unprocessed and processed *Citrullus lanatus* (Watermelon), *Moringa oleifera* (Moringa) and *Telfaira occidentalis* (Fluted pumpkin) seed flours are shown in Table 4.3a, 4.3b and 4.3c respectively.

Vitamin E was significantly higher ($p<0.05$) in soaked seed flour of watermelon (0.52 mg/100 g), vitamin C was significantly higher ($p<0.05$) in boiled watermelon seed flour 2.40 mg/100 as revealed in Table 4.3a, when compared to watermelon unprocessed seed. There was no significant ($p >0.05$) difference in the vitamin B, folic acid and vitamin K contents in all the processed seed flours of watermelon when compared to its unprocessed seed flour.

There was no significant ($p > 0.05$) difference seen (Table 4.3b) in the vitamin B_{12}, vitamin K, vitamin B_2 and vitamin D of the processed seed flours of moringa when compare to the unprocessed, the vitamin E of these group ranges from 0.13 mg/100 g in boiled moringa to 0.24 mg/100 g in soaked moringa seed flour.

There was significant different ($p<0.05$) in the vitamin E, vitamin C and vitamin D of the *Telfaira occidentalis* seed flours as shown in Table 4.3c.

Generally, in all the seeds there were no significant different ($p \geq 0.05$) in the vitamin B, vitamin K and folic acids when compare to the unprocessed groups.

Table 4.3a: Vitamin Content of Unprocessed and Processed *Citrullus lanatus* (Watermelon) Seed Flour

Vitamin Composition (mg/100g)	UWMSF	SWMSF	GWMSF	BWMSF
Vit. E	0.35 ± 0.03^{b}	0.52 ± 0.00^{c}	0.26 ± 0.00^{a}	0.25 ± 0.01^{a}
Vit. C	1.45 ± 0.01^{b}	1.13 ± 0.09^{a}	1.04 ± 0.01^{a}	2.40 ± 0.17^{c}
Vit. B12	1.12 ± 0.00^{c}	1.12 ± 0.00^{c}	0.76 ± 0.33^{b}	0.21 ± 0.00^{a}
Vit. D	0.01 ± 0.00^{a}	0.05 ± 0.00^{c}	0.01 ± 0.00^{a}	0.03 ± 0.00^{b}
Vit. B2	0.77 ± 0.34^{a}	0.81 ± 0.36^{a}	0.11 ± 0.01^{a}	0.15 ± 0.03^{a}
Vit. B6	0.12 ± 0.00^{b}	0.10 ± 0.00^{a}	0.12 ± 0.00^{b}	0.12 ± 0.00^{b}
Vit. K	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Folic acid	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SWMSF –Soaked Watermelon Seed Flour
GWMSF- Germinated Watermelon Seed Flour
BWMSF- Boiled Watermelon Seed Flour
UWMSF- Unprocessed Watermelon Seed Flour

Table 4.3b: Vitamin Content of Unprocessed and Processed *Moringa Oleifera* (Moringa) Seed Flour

Vitamin Composition (mg/100g)	UMFR	SMFR	GMFR	BMFR
Vit. E	0.22 ± 0.00^{b}	0.24 ± 0.00^{c}	0.13 ± 0.00^{a}	0.13 ± 0.01^{a}
Vit. C	2.33 ± 0.19^{c}	0.81 ± 0.01^{a}	1.08 ± 0.00^{b}	0.81 ± 0.01^{a}
Vit. B12	1.11 ± 0.00^{a}	1.13 ± 0.00^{a}	1.11 ± 0.00^{a}	1.11 ± 0.00^{a}
Vit. D	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.49 ± 0.14^{b}	0.03 ± 0.00^{a}
Vit. B2	0.47 ± 0.32^{a}	0.82 ± 0.34^{a}	0.78 ± 0.32^{a}	0.44 ± 0.33^{a}
Vit. B6	0.10 ± 0.00^{a}	0.12 ± 0.00^{b}	0.14 ± 0.00^{c}	0.17 ± 0.00^{d}
Vit. K	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{b}
Folic acid	0.00 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SMSF- Soaked Moringa seed flour
GMSF- Germinated Moringa seed flour
BMSF- Boiled Moringa seed flour
UMSF-Unprocessed Moringa seed flour

Table 4. 3c: Vitamin Content of Unprocessed and Processed *Telfaira occidentalis* (Fluted pumpkin) Seed Flour

Vitamin Composition (mg/100g)	UFSF	SFSF	GFSF	BFSF
Vit. E	0.16 ± 0.00^{b}	0.15 ± 0.00^{b}	0.13 ± 0.00^{a}	0.27 ± 0.01^{c}
Vit. C	2.36 ± 0.00^{c}	1.51 ± 0.01^{a}	1.70 ± 0.06^{b}	1.65 ± 0.01^{b}
Vit. B12	0.42 ± 0.00^{a}	0.14 ± 0.07^{a}	0.72 ± 0.00^{b}	0.78 ± 0.06^{b}
Vit. D	0.28 ± 0.00^{b}	0.01 ± 0.00^{a}	0.04 ± 0.00^{c}	0.02 ± 0.00^{b}
Vit. B2	0.19 ± 0.11^{a}	0.12 ± 0.05^{a}	0.39 ± 0.19^{a}	0.28 ± 0.21^{a}
Vit. B6	0.19 ± 0.00^{b}	0.17 ± 0.00^{b}	0.15 ± 0.03^{a}	0.21 ± 0.00^{c}
Vit. K	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Folic acid	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SFSF- Fluted Pumpkin Soaked Fluted Pumpkin Seed Flour
GFSF- Germinated Fluted Pumpkin Seed Flour BFSF-
Boiled Fluted Pumpkin Seed Flour UFSF- Unprocessed
Fluted Pumpkin Seed Flour

4.1.1.4 *Amino acids profile of unprocessed and processed Citrullus lanatus, Moringa oleifera and Telfaira occidentalis seed flours*

The amount of amino acids present in the processed and unprocessed seeds of *Citrullus lanatus, Moringa oleifera and Telfaira occidentalis* were shown in Table 4.4a, 4.4b and 4.4c respectively.

Significant ($p< 0.05$) increase in histidine, threonine, valine, lysine, leucine, isoleucine and phenyalanine levels was showed in soaked seed flour of watermelon (Table 4.4a) when compared to its unprocessed seed flour. No significant ($p>0.05$) difference was seen in arginine, methionine, proline and aspartic acid levels in all seed flours of watermelon.

No significant ($p>0.05$) difference was revealed in histidine, valine, cysteine, and glutamic acid of the various seed flours of moringa as shown in Table 4.4b . Isoleucine and lysine were significantly ($p<0.05$) higher in the soaked and boiled seed flours of moringa respectively when compared to the unprocessed moringa seed flour.

Significant ($p<0.05$) different was shown in the amino acid content in processed and unprocessed fluted pumpkin seed flours (Table 4.4c). Histidine was higher in the unproceesed seed flour of fluted pumpkin when compared to the processed seed flours. Leucine, lycine, tryptophan, valine, arginine, proline, alanine, glutamic acid and aspartic acid levels were higher in soaked seed of fluted pumpkin when compared to its unprocessed seed flour.

Table 4.4a: Amino Acids Profile of Unprocessed and Processed *Citrullus lanatus* (Watermelon) Seed Flours

Amino acids (g/100g)	UWMSF	SWMSF	GWMSF	BWMSF	FAOWHO (1993)
Histidine	2.17±0.02^{c}	2.24 ± 0.01^{d}	1.85±0.03^{a}	2.00±0.01^{b}	2.4
Isoleucine	3.01±0.01^{b}	3.21±0.12^{b}	2.70±0.17^{a}	2.81±0.06^{a}	4.2
Leucine	5.84±0.06^{b}	6.10±0.06^{b}	5.60±0.17^{a}	5.81±0.12^{b}	4.9
Lysine	4.45±0.03^{b}	4.75±0.12^{c}	4.03±0.02^{a}	3.92±0.02^{a}	4.2
Methionine	2.13±0.02^{a}	2.20±0.12^{a}	2.00±0.29^{a}	2.30±0.17^{a}	2.2
Phenylalanine	3.72±0.02^{b}	4.00±0.29^{c}	3.37±0.06^{a}	3.10±0.06^{a}	2.8
Threonine	3.55±0.03^{a}	4.22±0.01^{c}	3.83±0.02^{b}	3.61±0.06^{a}	4.0
Tryptophan	1.07±0.02^{b}	0.93±0.02^{a}	1.23±0.05^{c}	1.05±0.03^{b}	
Valine	3.71±0.06^{a}	4.62±0.01^{b}	4.10±0.17^{a}	3.75±0.19^{a}	4.2
Arginine	10.20±0.15^{a}	10.83±0.06^{a}	10.16±0.58^{a}	10.41±0.01^{a}	2.0
Cysteine	1.94±0.02^{a}	2.42±0.01^{c}	2.36±0.06^{c}	2.24±0.02^{b}	
Glycine	3.85±0.03^{b}	5.37±0.02^{d}	4.82±0.01^{c}	3.66±0.06^{a}	
Proline	4.60±0.23 ^{a}	4.90±0.23^{a}	5.00±0.12^{a}	4.50±0.12^{a}	
Serine	4.00±0.58^{a}	3.70±0.12^{a}	3.35±0.06^{a}	3 . 2 0 ± 0 . 1 2 ^{a}	
Tyrosine	2.10±0.06^{a}	2.41±0.02^{b}	2.06±0.01^{a}	2 . 4 1 ± 0 . 0 1 ^{b}	4.1
Alanine	4.52±0.15 ^{c}	3.37±0.06^{a}	3.56±0.01^{a}	3.87±0.06^{b}	
Glutamic acid	15.75±0.03^{c}	15.61±0.01^{b}	15.29±0.06^{a}	16.65±0.03^{d}	
Aspartic acid	8.75±0.03 ^{a}	8.00±1.15^{a}	8.68±0.01^{a}	8 . 9 9 ± 0 . 0 1 ^{a}	4.0

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SWMSF –Soaked Watermelon Seed Flour
GWMSF- Germinated Watermelon Seed Flour
BWMSF- Boiled Watermelon Seed Flour
UWMSF- Unprocessed Water melon Seed Flour

Table 4.4b: Amino Acids Profile of Unprocessed and Processed *Moringa oleifera* (Moringa) Seed Flours

Amino acids (g/100g)	UMFR	SMFR	GMFR	BMFR	FAO/WHO (1993)
Histidine	2.30±0.06^{a}	2.30±0.17^{a}	2.24±0.01^{a}	2.23±0.02^{a}	2.4
Isoleucine	2.03±0.01^{a}	2.30±0.02^{c}	2.16±0.02^{b}	2.43±0.06^{d}	4.2
Leucine	7.30±0.06^{b}	6.90±0.06^{a}	7.00±0.29^{a}	7.59±0.03^{b}	4.9
Lysine	4.67±0.01^{c}	4.08±0.02^{a}	4.38±0.09^{b}	5.04±0.02^{d}	4.2
Methionine	1.44±0.06^{b}	1.30±0.06^{a}	1.39±0.01^{b}	1.50±0.06^{b}	2.2
Phenylalanine	4.52±0.06^{b}	4.26±0.02^{b}	3.84±0.32^{a}	3.99±0.01^{a}	2.8
Threonine	4.00±0.12^{b}	3.99±0.01^{b}	3.72±0.01^{a}	4.33±0.06^{c}	4.0
Tryptophan	1.16±0.01^{b}	1.05±0.01^{a}	1.15±0.03^{b}	1.37±0.02^{c}	
Valine	4.50±0.17^{a}	4.00±0.58^{a}	4.38±0.06^{a}	4.33±0.02^{a}	4.2
Arginine	7.48±0.06^{c}	7.05±0.03^{b}	7.74±0.02^{d}	6.62±0.01^{a}	2.0
Cysteine	2.18±0.01^{a}	4.33±1.68^{a}	1.82±0.01^{a}	2.42±0.01^{a}	
Glycine	5.23±0.02^{d}	5.01±0.01^{c}	4.32±0.02^{a}	4.94±0.01^{b}	
Proline	4.97±0.01^{c}	4.70±0.12^{b}	4.47±0.01^{a}	5.28±0.01^{d}	
Serine	3.29±0.01^{b}	3.45±0.01^{c}	3.08±0.02^{a}	3.83±0.03^{d}	
Tyrosine	2.06±0.02^{c}	1.90±0.06^{b}	1.63±0.02^{a}	2.58±0.01^{d}	4.1
Alanine	4.52±0.15^{c}	3.37±0.06^{a}	3.56±0.01^{a}	3.87±0.06^{b}	
Glutamic acid	8.75±0.03^{a}	8.00±1.15^{a}	8.68±0.01^{a}	8.99±0.01^{a}	
Aspartic acid	15.75±0.03^{c}	15.61±0.01^{b}	15.29±0.06^{a}	16.65±0.03^{d}	4.0

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SMSF- Soaked Moringa seed flour
GMSF- Germinated Moringa seed flour
BMSF- Boiled Moringa seed flour
UMSF-Unprocessed Moringa seed flour

Table 4.4c: Amino Acids Profile of Unprocessed and Processed *Telfaira occidentalis* (Fluted Pumpkin) Seed Flours

Amino acids(g/100g)	UFSF	SFSF	GFSF	BFSF	FAO/WHO (1993)
Histidine	2.36±0.02^{d}	2.24±0.02^{c}	2.11±0.01^{a}	2.17±0.01^{b}	2.4
Isoleucine	3.01±0.01^{c}	3.01±0.01^{c}	2.36±0.01^{b}	2.23±0.02^{a}	4.2
Leucine	7.00±0.29^{b}	7.18±0.01^{c}	6.36±0.06^{a}	6.59±0.06^{a}	4.9
Lysine	3.45±0.06^{a}	4.61±0.06^{d}	3.71±0.02^{b}	4.03±0.02^{c}	4.2
Methionine	1.55±0.06^{b}	1.49±0.01^{a}	1.60±0.06^{c}	1.71±0.02^{d}	2.2
Phenylalanine	3.37±0.06^{a}	3.90±0.06^{b}	3.90±0.06^{b}	4.26±0.01^{c}	2.8
Threonine	3.61±0.01^{a}	4.50±0.65^{b}	3.22±0.01^{a}	5.55±0.03^{c}	4.0
Tryptophan	1.26±0.01^{a}	1.58±0.01^{c}	1.31±0.01^{b}	1.26±0.01^{a}	
Valine	3.22±0.01^{a}	3.92±0.01^{d}	3.62±0.03^{c}	3.51±0.01^{b}	4.2
Arginine	8.95±0.01^{a}	10.66±0.02^{d}	9.81±0.01^{b}	9.98±0.01^{c}	2.0
Cysteine	2.30±0.17^{a}	2.30±0.17^{a}	2.18±0.01^{a}	1.94±0.02^{a}	
Glycine	3.94±0.02^{a}	4.89±0.01^{b}	5.61±0.01^{c}	5.56±0.02^{c}	
Proline	3.25±0.06^{a}	5.07±0.02^{d}	4.26±0.01^{c}	3.96±0.01^{b}	
Serine	3.24±0.02^{a}	4.00±0.58^{a}	3.62±0.01^{a}	3.56±0.06^{a}	
Tyrosine	1.55±0.03^{b}	1.20±0.12^{a}	1.29±0.01^{a}	1.55±0.01^{b}	4.1
Alanine	4.02±0.01^{b}	4.32±0.01^{c}	3.72±0.01^{a}	3.87±0.02^{a}	
Glutamic acid	14.61±0.06^{c}	14.89±0.01^{d}	13.85±0.03^{a}	14.00±0.58^{b}	
Aspartic acid	8.56±0.03^{a}	8.93±0.02^{b}	8.31±0.01^{a}	8.50±0.29^{a}	4.0

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SFSF- Fluted Pumpkin Soaked Fluted Pumpkin Seed Flour
GFSF- Germinated Fluted Pumpkin Seed Flour BFSF-
Boiled Fluted Pumpkin Seed Flour
UFSF- Unprocessed Fluted Pumpkin Seed Flour

4.1.2 Antinutrient composition of unprocessed and processed *Citrullus lanatus*, *Moringa oleifera* and *Telfaira occidentalis* seed Flours

The antinutrients composition of both unprocessed and processed *Citrullus lanatus* (Watermelon), *Moringa oleifera* (Moringa) and *Telfaira occidentalis* (Fluted pumpkin) seeds were shown in Table 4.5a, 4.5b, 4.5c respectively. There was significant ($p< 0.05$) different in the antinutrients composition of all the seed flours at different processing methods when compared to their individual unprocessed seed flours. The result showed that tannins, phytate, oxalate, saponins, trypsin inhibitor and cyanogenic glycoside was higher only in the three unprocessed seed flours but lower in the processed seed flours.

Table 4.5a: Antinutrient Composition of Unprocessed and Processed Citrullus *lanatus* (Watermelon) Seed Flour

Antinutrient Composition (mg/100g)	UWMSF	SWMSF	GWMSF	BWMSF
Tannins	33.68 ± 0.32^{d}	18.92 ± 0.13^{a}	31.40 ± 0.03^{c}	27.92 ± 0.11^{b}
Phytate	0.36 ± 0.01^{d}	0.12 ± 0.01^{b}	0.29 ± 0.01^{c}	0.11 ± 0.01^{a}
Oxalate	0.23 ± 0.01^{d}	0.18 ± 0.01^{c}	0.14 ± 0.01^{a}	0.17 ± 0.00^{b}
Saponins(g/100g)	3.46 ± 0.03^{c}	2.45 ± 0.03^{b}	2.32 ± 0.04^{b}	0.64 ± 0.01^{a}
T I	0.07 ± 0.01^{c}	0.06 ± 0.20^{b}	0.06 ± 0.01^{b}	0.05 ± 0.02^{a}
CG	1.99 ± 0.01^{c}	1.37 ± 0.01^{b}	0.71 ± 0.32^{a}	0.71 ± 0.06^{a}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$) TI - Trypsin Inhibitor, CG- Cyanogenic Glycoside
SWMSF –Soaked Watermelon Seed Flour
GWMSF- Germinated Watermelon Seed Flour
BWMSF- Boiled Watermelon Seed Flour
UWMSF- Unprocessed Watermelon Seed Flour

Table 4.5b: Antinutrient Composition of Unprocessed and Processed *Moringa oleifera* (Moringa) Seed Flour

Antinutrient Composition (mg/100g)	UMSF	SMSF	GMSF	BMSF
Tannins	36.90 ± 0.29^{c}	28.27 ± 0.11^{b}	15.74 ± 0.08^{a}	27.92 ± 0.11^{b}
Phytate	0.35 ± 0.01^{c}	0.16 ± 0.01^{b}	0.14 ± 0.01^{a}	0.11 ± 0.01^{a}
Oxalate	0.73 ± 0.01^{c}	0.64 ± 0.01^{b}	0.58 ± 0.01^{b}	0.17 ± 0.00^{a}
Saponins(g/100g)	2.99 ± 0.01^{d}	2.03 ± 0.01^{a}	2.27 ± 0.01^{b}	2.76 ± 0.02^{c}
T I	0.09 ± 0.01^{b}	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}	0.05 ± 0.02^{a}
CG	1.68 ± 0.01^{d}	1.55 ± 0.02^{c}	1.33 ± 0.02^{b}	0.09 ± 0.01^{a}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$) TI - Trypsin Inhibitor, CG- Cyanogenic Glycoside
SMSF- Soaked Moringa seed flour
GMSF- Germinated Moringa seed flour
BMSF- Boiled Moringa seed flour
UMSF-Unprocessed Moringa seed flour

Table 4.5c: Antinutrient Composition of Unprocessed and Processed *Telfaira occidentalis* (Fluted pumpkin) Seed Flour

Antinutrient Composition (mg/100g)	UFSF	SFSF	GFSF	BFSF
Tannins	21.91 ± 0.06^{d}	9.57 ± 0.15^{a}	18.87 ± 0.09^{c}	12.69 ± 0.12^{b}
Phytate	0.42 ± 0.01^{c}	0.35 ± 0.00^{b}	0.30 ± 0.01^{a}	0.28 ± 0.01^{a}
Oxalate	0.22 ± 0.01^{c}	0.15 ± 0.01^{a}	0.18 ± 0.01^{b}	0.16 ± 0.01^{a}
Saponins(g/100g)	1.85 ± 0.03^{c}	0.27 ± 0.01^{a}	1.32 ± 0.01^{b}	0.26 ± 0.01^{a}
T I	0.05 ± 0.00^{b}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}
CG	4.26 ± 0.02^{d}	1.63 ± 0.03^{a}	4.01 ± 0.04^{c}	2.16 ± 0.02^{b}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
TI - Trypsin Inhibitor, CG- Cyanogenic Glycoside SFSF- Fluted Pumpkin Soaked Fluted Pumpkin Seed Flour
GFSF- Germinated Fluted Pumpkin Seed Flour BFSF- Boiled Fluted Pumpkin Seed Flour
UFSF- Unprocessed Fluted Pumpkin Seed Flour

4.1.3: Functional properties of unprocessed and processed *Citrullus lanatus*, *Moringa oleifera* and *Telfaira occidentalis*

The solubility of the processed and unprocessed seeds of watermelon, moringa and fluted pumpkin at 60 ^{0}C, 70 ^{0}C, 80 ^{0}C, 90 ^{0}C and 100 ^{0}C are shown in Figure 4.1a, 4.1b, 4.1c respectively.

Soaked seed flour of watermelon has the highest level of solubility at 60 ^{0}C and 100 ^{0}C when compared to the unprocessed seed flour, the solubility in boiled and germinated watermelon seed flour were observed to be constant at 70 ^{0}C, 80 ^{0}C and 90 ^{0}C (Figure 4.1a). There is significant ($p<0.05$) difference in the solubility rate of the seed flours of moringa at different level of temperature (Figure 4.1b). Soaked seed flour of fluted pumpkin show significant ($p<0.05$) increase in solubility rate at 60 ^{0}C, 70 ^{0}C, and 100 ^{0}C respectively (Figure 4.1c).

The swelling capacity in the processed and unprocessed seeds of watermelon, moringa and fluted pumpkin respectively at 60 ^{0}C, 70 ^{0}C, 80 ^{0}C, 90 ^{0}C and 100 ^{0}C are shown in Figure 4.2a, 4.2b, 4.2c respectively. There is significant ($p < 0.05$) difference in all seed flours of watermelon at different levels of temperature as seen in Figure 4.2a. The boiled seed flour of moringa showed the highest swelling capacity of (4.70 ± 0.12) at 90 ^{0}C while the soaked seed flour of moringa show the lowest of (2.75 ± 0.12) at 60 ^{0}C (Figure 4.2b). there is significant ($p < 0.05$) difference in all seed flours of fluted pumpkin at different levels of temperature with soaked seed flour of fluted pumpkin showing increased in swelling capacity at 60 ^{0}C, 70 ^{0}C and 100 ^{0}C (Figure 4.2c).

Water absorption capacity (WAC), oil absorption capacity (OAC), bulk density (BD), emulsions capacity (EC) and foaming capacity (FC) in the processed and unprocessed seeds of *Citrullus lanatus, Moringa oleifera Lam.* and *Telfaira occidentalis* are shown in Table 4.6a, 4.6b, 4.6c respectively.

There was significant ($p < 0.05$) difference in WAC, BD and FC as revealed in Table 4.6a. WAC was higher in unprocessed seed flour of *Citrullus* showed that *lanatus*, no significant ($p>0.05$) difference seen in OAC of soaked and germinated seeds of watermelon when compared to the unprocessed seed flour and significant ($p<0.05$) difference seen in EC, ES, and FS of processed seed flours of *Citrullus lanatus* when compared to the unprocessed. Significant ($p<0.05$) difference was seen in WAC, BD, FC, FS, EC, ES of all the processed seed flour of *Moringa oleifera* when compared to it unprocessed seed flour(Table 4.6b). There was significant ($p<0.05$) difference in WAC, OAC, BD, EC and FC of the processed seed flours of *Telfaira occidentalis* when compared to it unprocessed seed flour (Table 4.6c).

On a general note WAC ranges from 17.65 % in boiled seed flour of moringa to 53.85 % in unprocessed seed flour of watermelon, OAC was generally lower in seed flour of *Telfaira occidentalis* when compared to the value obtained in other seed flours.

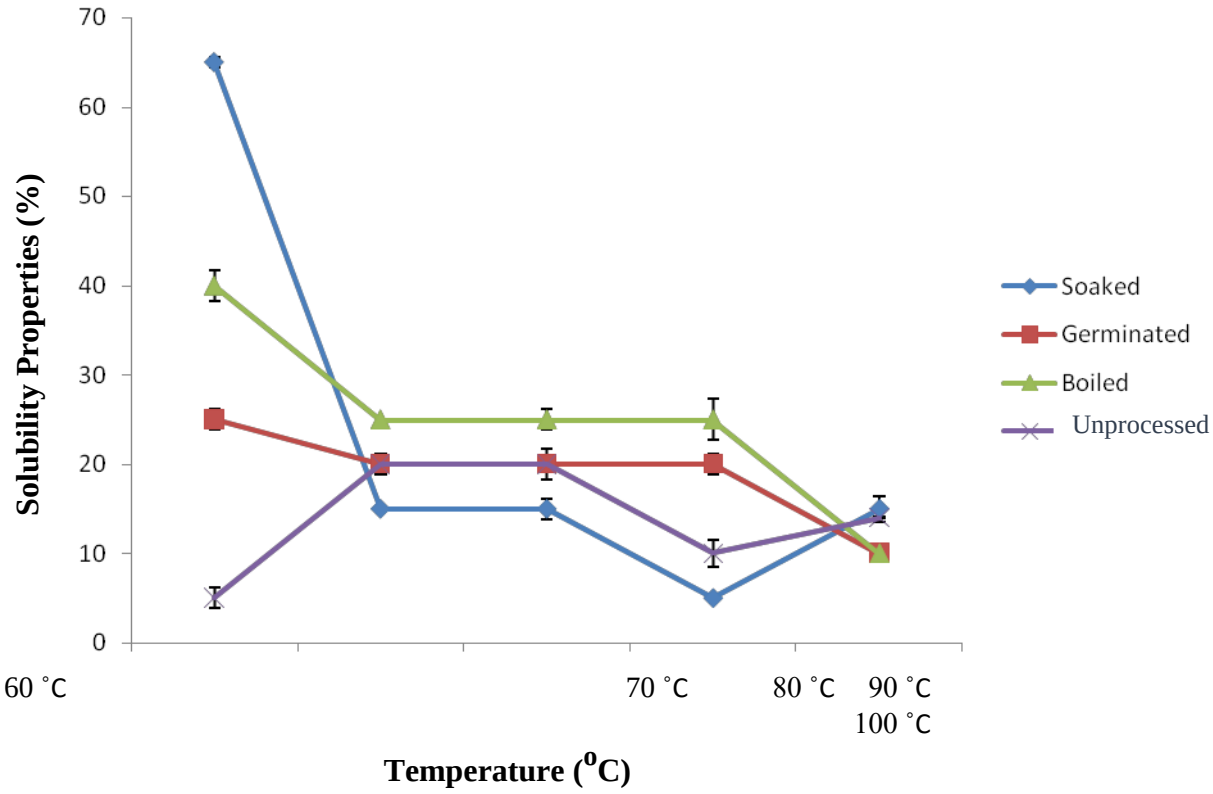


Figure 4.1a: Solubility Properties of Unprocessed and Processed *Citrullus lanatus* (Watermelon) Seed Flour

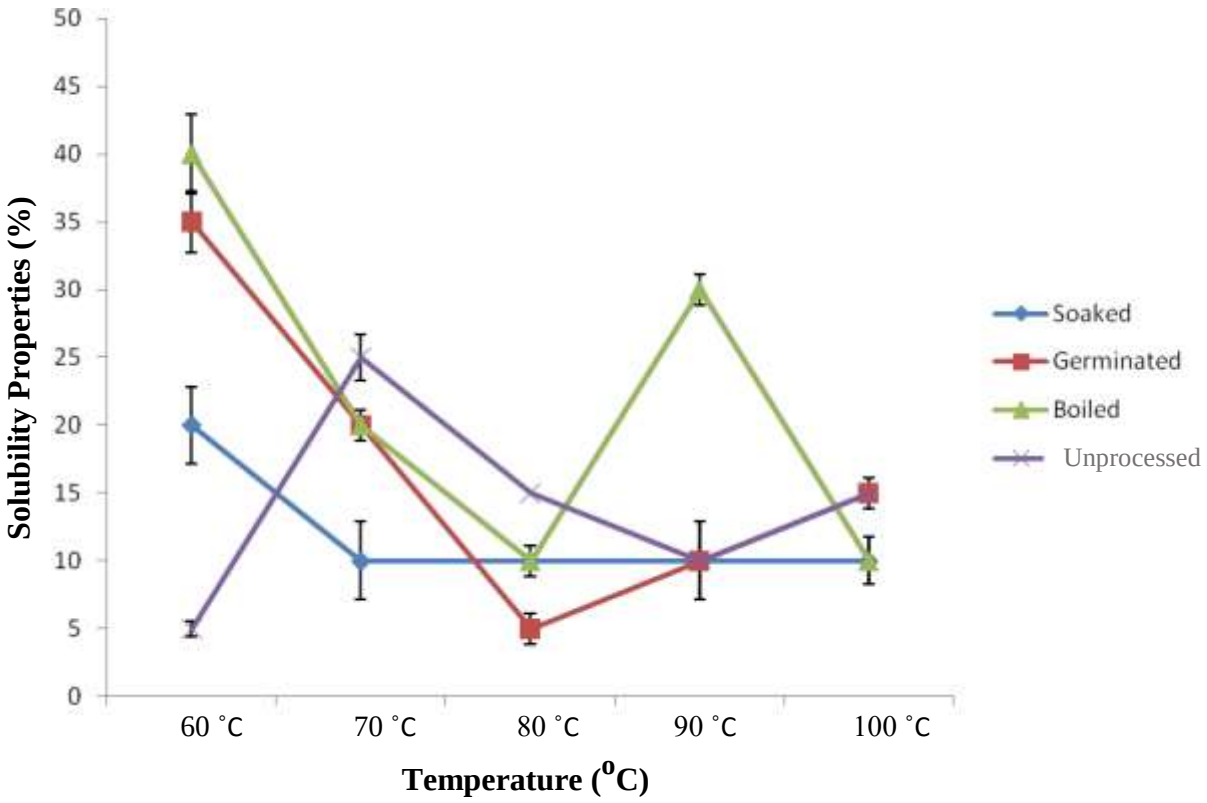


Figure 4.1b: Solubility Properties of Unprocessed and Processed *Moringa oleifera* (Moringa) Seed Flour

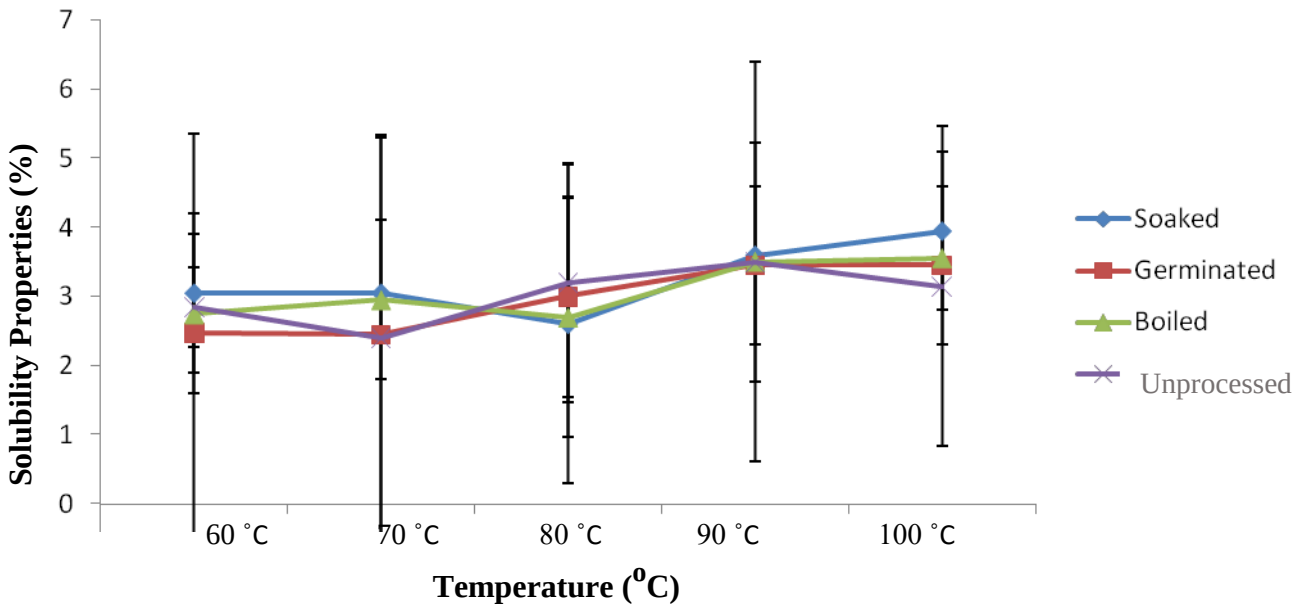


Figure 4.1c: Solubility Properties of Unprocessed and Processed *Telfaira occidentalis* (fluted pumpkin) Seed Flour

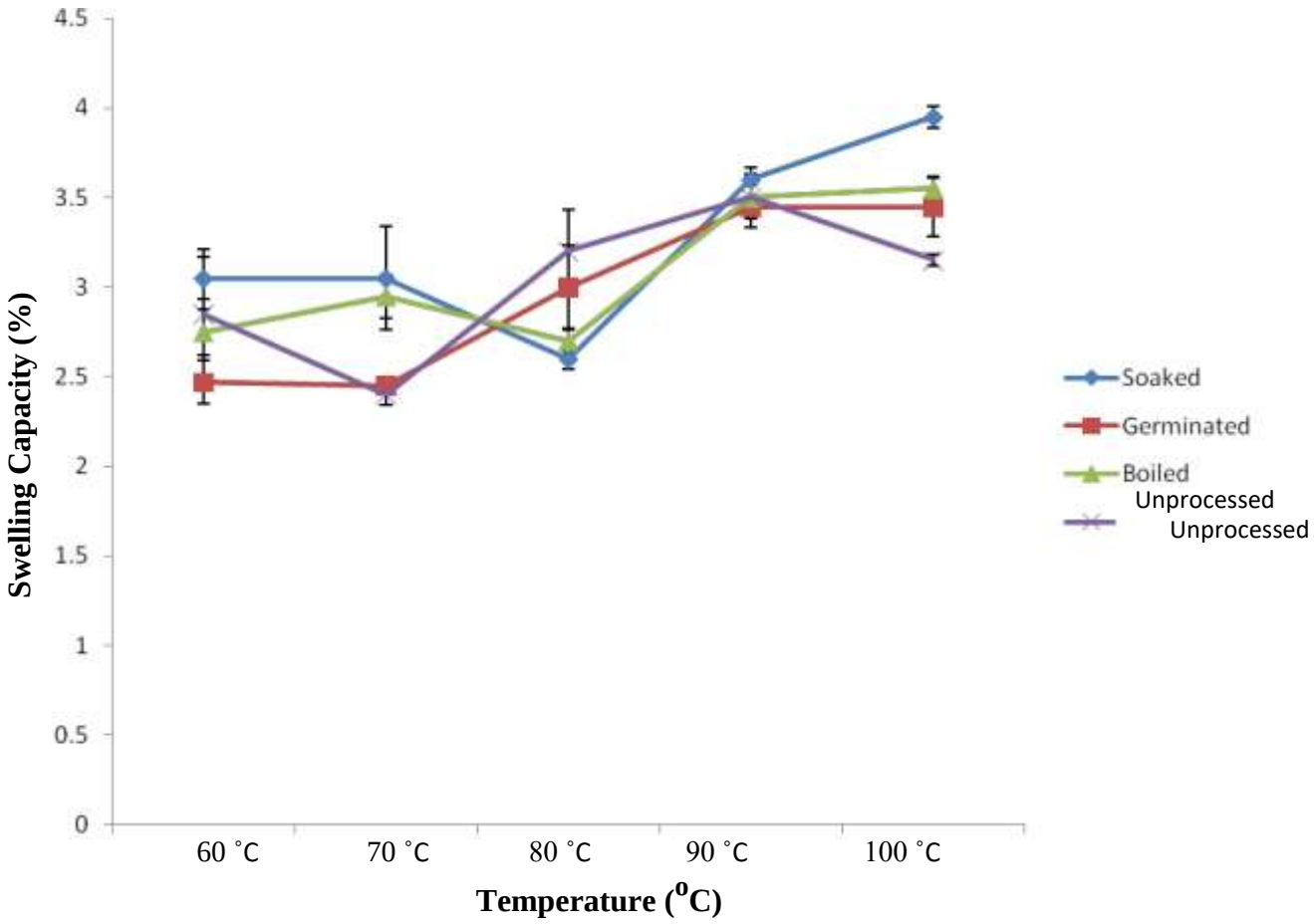


Figure 4.2a: Swelling Capacity of Unprocessed and Processed *Citrullus lanatus* (Watermelon) Seed Flour

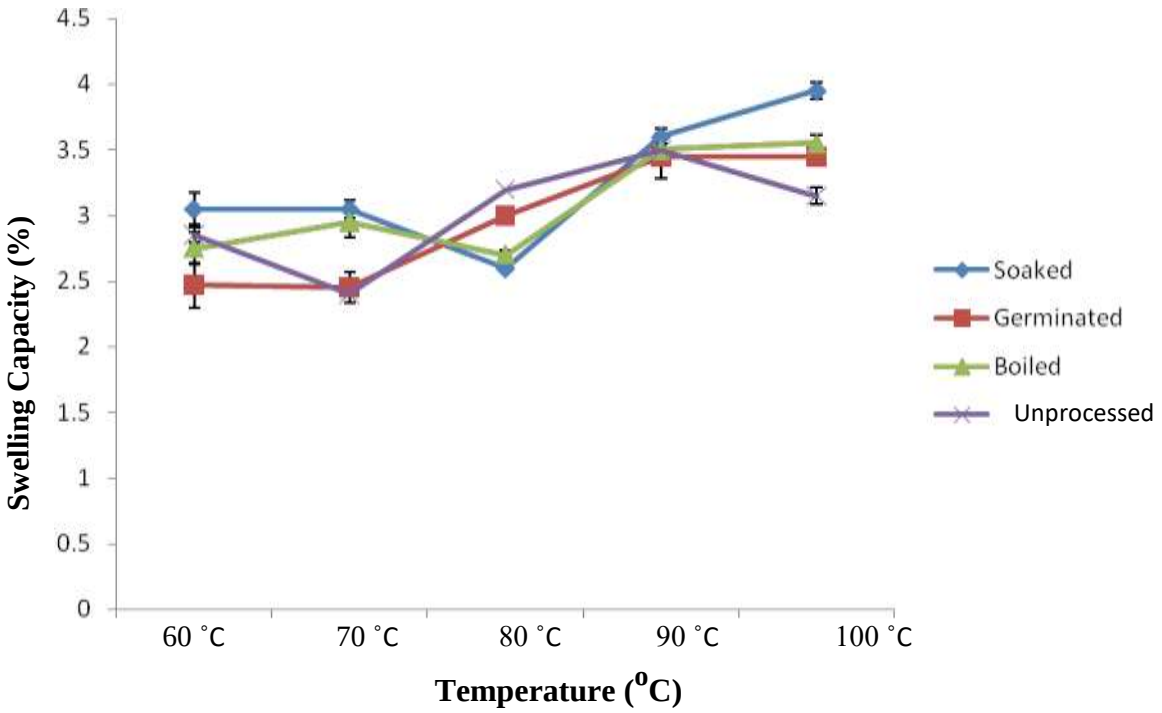


Figure 4.2b: Swelling Capacity of Unprocessed and Processed *Moringa oleifera* (Moringa) Seed Flour

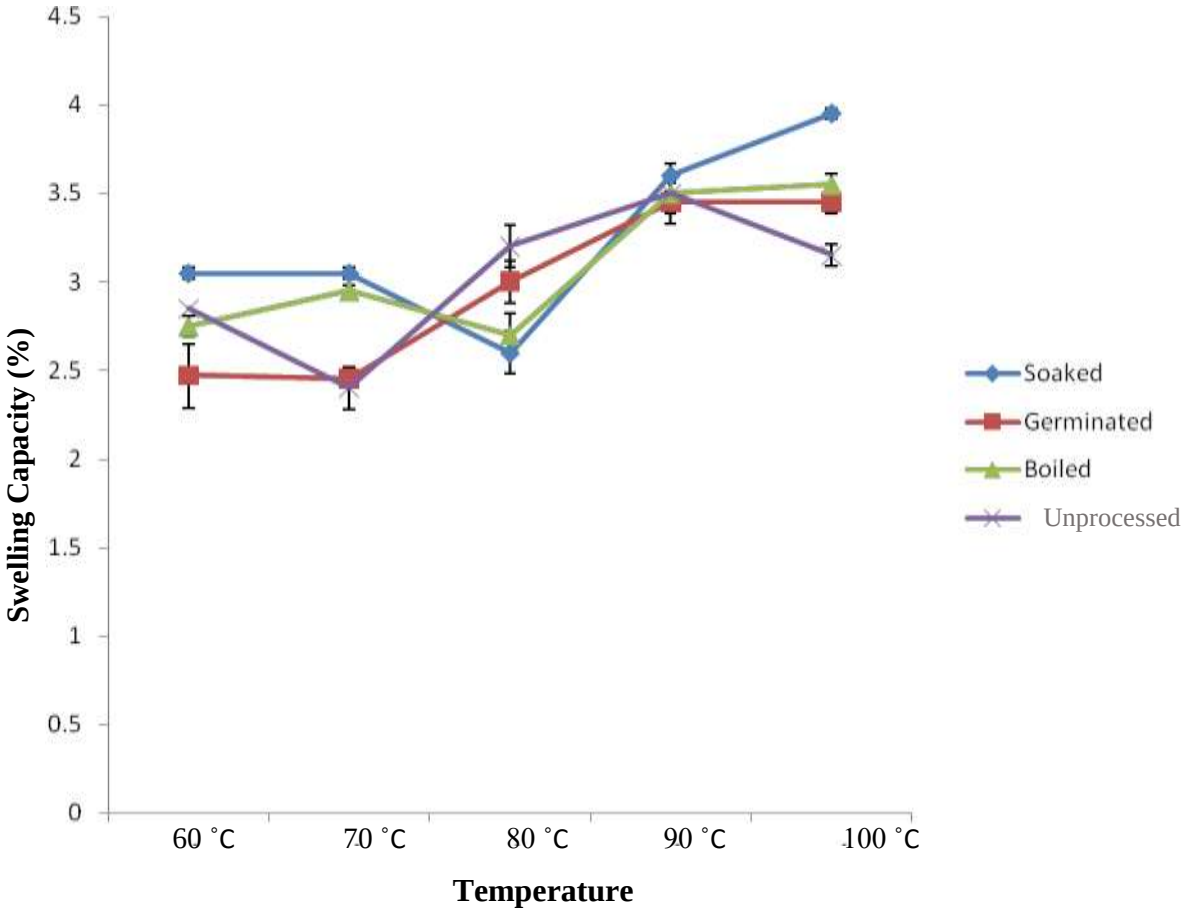


Figure 4.2c: Swelling Capacity of Unprocessed and Processed *Telfaira occidentalis* (Fluted Pumpkin) Seed Flour

Table 4.6a: Functional Properties of Unprocessed and Processed *Citrullus lanatus* (Watermelon) Seed Flour

Functional Properties (%)	UWMSF	SWMSF	GWMSF	BWMSF
BD(g/ml)	0.48 ± 0.21^{a}	0.63 ± 0.21^{b}	0.59 ± 0.14^{b}	0.67 ± 0.21^{b}
WAC	53.85 ± 0.82^{b}	33.33 ± 0.82^{a}	33.33 ± 0.75^{a}	33.33 ± 0.78^{a}
OAC	33.33 ± 0.05^{a}	33.33 ± 0.05^{a}	33.33 ± 0.13^{a}	42.86 ± 0.03^{b}
FC	6.67 ± 0.32^{d}	1.96 ± 0.39^{b}	1.96 ± 0.18^{a}	2.86 ± 0.36^{c}
FS	57.14 ± 0.04^{c}	50.37 ± 0.35^{b}	50.37 ± 0.01^{a}	66.66 ± 0.07^{d}
EC	26.66 ± 0.67^{a}	44.00 ± 0.65^{c}	43.33 ± 0.47^{c}	33.33 ± 0.35^{b}
ES	30.05 ± 0.49^{b}	16.66 ± 0.49^{a}	46.15 ± 0.48^{d}	40.21 ± 0.32^{c}

Values are expressed as means of triplicate determinations ± SEM.Values along rows with different superscript are significantly different ($p<0.05$)
WAC: water absorption capacity, OAC: oil absorption capacity, BD: bulk density, EC: emulsion capacity, FC: foaming capacity, FS: foaming stability, ES: emulsion stability
SWMSF –Soaked Watermelon Seed Flour
GWMSF- Germinated Watermelon Seed Flour
BWMSF- Boiled Watermelon Seed Flour
UWMSF- Unprocessed Watermelon Seed Flour

Table 4.6b: Functional Properties of Unprocessed and Processed *Moringa oleifera* (Moringa) Seed Flour

Functional Properties (%)	UMFR	SMFR	GMFR	BMFR
BD(g/ml)	0.56 ± 0.03^{b}	0.48 ± 0.06^{a}	0.56 ± 0.02^{b}	0.63 ± 0.15^{b}
WAC	33.33 ± 0.64^{c}	33.33 ± 0.74^{c}	25.22 ± 0.64^{b}	17.65 ± 0.73^{a}
OAC	42.86 ± 0.55^{c}	42.86 ± 0.59^{c}	33.33 ± 0.5^{b}	25.20 ± 0.64^{a}
FC	6.67 ± 0.57^{c}	4.76 ± 0.62^{b}	3.81 ± 0.66^{a}	8.57 ± 0.52^{d}
FS	85.71 ± 0.22^{b}	91.37 ± 0.15^{d}	75.19 ± 0.29^{a}	88.80 ± 0.12^{c}
EC	36.66 ± 0.74^{d}	33.33 ± 0.55^{c}	26.66 ± 0.67^{b}	16.61 ± 0.60^{a}
ES	36.36 ± 0.63^{a}	80.66 ± 0.74^{d}	50.01 ± 0.64^{c}	40.01 ± 0.26^{b}

Values are expressed as means of triplicate determinations ± SEM.Values along rows with different superscript are significantly different ($p< 0.05$)
WAC: water absorption capacity, OAC: oil absorption capacity, BD: bulk density, EC: emulsion capacity, FC: foaming capacity, FS: foaming stability, ES: emulsion stability
SMSF- Soaked Moringa seed flour
GMSF- Germinated Moringa seed flour
BMSF- Boiled Moringa seed flour
UMSF-Unprocessed Moringa seed flour

Table 4.6c: Functional Properties of Unprocessed and Processed*Telfaira occidentalis* (Fluted pumpkin) Seed Flour

Functional Properties (%)	UFSF	SFSF	GFSF	BFSF
BD(g/ml)	0.67 ± 0.16^{a}	0.67 ± 0.06^{a}	0.71 ± 0.02^{b}	0.67 ± 0.01^{a}
WAC	$25.00\pm0,41^{a}$	42.86 ± 034^{d}	33.33 ± 0.39^{b}	25.00 ± 0.32^{a}
OAC	5.26 ± 0.24^{a}	25.00 ± 0.47^{c}	5.26 ± 0.32^{a}	11.71 ± 0.40^{b}
FC	2.86 ± 0.08^{a}	6.67 ± 0.15^{c}	4.76 ± 0.29^{b}	2.86 ± 0.07^{a}
FS	66.66 ± 0.74^{a}	71.42 ± 0.54^{b}	80.09 ± 0.46^{c}	66.66 ± 0.60^{a}
EC	26.66 ± 0.35^{a}	40.00 ± 0.38^{b}	40.00 ± 0.56^{b}	40.00 ± 0.64^{b}
ES	75.00 ± 0.66^{c}	50.00 ± 0.59^{a}	66.15 ± 0.74^{b}	83.33 ± 0.42^{d}

Values are expressed as means of triplicate determinations ± SEM.Values along rows with different superscript are significantly different ($p<0.05$)
WAC: water absorption capacity, OAC: oil absorption capacity, BD: bulk density, EC: emulsion capacity, FC: foaming capacity, FS: foaming stability, ES: emulsion stability.
SFSF- Fluted Pumpkin Soaked Fluted Pumpkin Seed Flour GFSF- Germinated Fluted Pumpkin Seed Flour
BFSF- Boiled Fluted Pumpkin Seed Flour
UFSF- Unprocessed Fluted Pumpkin Seed Flour

4.1.4 Proximate Composition of supplemented diets formulated with unprocessed and processed seed flours of *Citrullus lanatus, Moringa oleifera* and *Telfaira occidentalis*

The proximate composition of experimental diets formulated with the processed and unprocessed seed flours of watermelon, moringa and fluted pumpkin shown in Tables 4.7a, 4.7b, and 4.7c respectively.

There was significant ($p<0.05$) difference in ash, moisture, fat, carbohydrate and fiber contents of all the processed seed flours supplemented diet of watermelon except for crude protein when compared to their unprocessed seed flour as revealed in Table 4.7a. The carbohydrate content of the diets ranged from 46.03±1.44 % in the control diet to 68.59±0.38 % in boiled watermelon diet. Unprocessed watermelon supplemented diet had the highest amount of crude fiber (19.20±0.74 %) in this group.

There no significant ($p>0.05$) difference seen in the crude protein content of all the moringa supplemented diet, soaked and germinated moringa seed flour supplemented diet (Table 4.7b). Germinated moringa seed flour supplemented diet have the highest carbohydrate contents.

Unprocessed fluted pumpkin diet had the least amount of crude fiber (1.07±0.22 %) while there was significant ($p<0.05$) difference in the metabolized energy, it ranges from 379.13 ± 0.6 Kcal/100 g in the control diet to 441.22 ± 0.51 Kcal/100 g in the boiled *Telfaira occidentalis* diet as represented in Table 4.7c.

Table 4.7a: Proximate Composition of Supplemented Diets Formulated with Unproccessed and Processed Seed Flours of *Citrullus lanatus*

Proximate Composition (%)	Control	UWMSF	SWMSF	GWMSF	BWMSF
Ash	15.50 ± 0.29^{c}	7.47 ± 0.34^{b}	8.2 ± 0.12^{b}	7.68 ± 0.4^{b}	3.6 ± 0.35^{a}
Moisture	8.47 ± 0.58^{c}	0.50 ± 0.06^{a}	0.55 ± 0.06^{a}	1.82 ± 0.26^{b}	2.20 ± 0.12^{b}
Fat	15.0 ± 0.58^{b}	10.25 ± 0.58^{a}	11.07 ± 0.64^{a}	10.6 ± 0.57^{a}	10.58 ± 0.87^{a}
Protein	15.01 ± 0.01^{a}	15.06 ± 0.04^{a}	15.03 ± 0.04^{a}	15.0 ± 0.23^{a}	15.06 ± 0.07^{a}
Carbohydrates	43.03 ± 1.44^{a}	47.53 ± 1.00^{bc}	56.85 ± 0.44^{b}	46.43 ± 0.54^{b}	57.15 ± 0.38^{c}
Fibre	3.00 ± 0.32^{a}	19.20 ± 0.74^{d}	8.30 ± 0.43^{b}	18.45 ± 0.29^{d}	11.44 ± 0.60^{c}
Energy (Kcal)	367.16 ± 0.6^{b}	342.61 ± 0.5^{a}	387.15 ± 0.87^{c}	341.12 ± 0.20^{a}	384.06 ± 6.19^{d}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$) SWMSF –Soaked Watermelon Seed Flour
GWMSF- Germinated Watermelon Seed Flour
BWMSF- Boiled Watermelon Seed Flour
UWMSF- Unprocessed Watermelon Seed Flour

Table 4.7b: Proximate Composition of Supplemented Diets Formulated with Unprocessed and Processed Seed Flours of *Moringa oleifera*

Proximate Composition (%)	Control	UMFR	SMFR	GMFR	BMFR
Ash	15.50 ± 0.29^{c}	6.65 ± 0.6^{b}	5.6 ± 0.81^{b}	2.5 ± 0.29^{a}	7.2 ± 0.11^{b}
Moisture	8.47 ± 0.58^{c}	0.85 ± 0.06^{a}	1.00 ± 0.06^{a}	2.55 ± 0.11^{b}	0.75 ± 0.14^{a}
Fat	15.0 ± 0.58^{b}	10.20 ± 0.53^{a}	10.6 ± 0.35^{a}	11.57 ± 0.55^{ab}	13.00 ± 0.58^{b}
Protein	15.01 ± 0.01^{a}	15.03 ± 0.03^{a}	15.04 ± 0.01^{a}	15.01 ± 0.01^{a}	15.04 ± 0.02^{a}
Carbohydrates	43.03 ± 1.44^{a}	55.06 ± 1.18^{b}	55.92 ± 1.22^{c}	56.41 ± 0.94^{c}	51.61 ± 0.89^{b}
Fibre	3.00 ± 0.32^{a}	12.24 ± 1.04^{b}	11.87 ± 0.32^{b}	11.97 ± 0.55^{b}	12.37 ± 0.42^{b}
Energy (Kcal)	367.16 ± 0.6^{b}	372.16 ± 0.11^{c}	379.24 ± 1.73^{d}	389.81 ± 4.34^{d}	358.93 ± 1.84^{a}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p< 0.05$) SMSF- Soaked Moringa seed flour

GMSF- Germinated Moringa seed flour
BMSF- Boiled Moringa seed flour
UMSF-Unprocessed Moringa seed flour

Table 4.7c: Proximate Composition of Supplemented Diets Formulated with Unproccessed and Processed Seed Flours of *Telfaira occidentalis*

Sample (%)	Control	UFSF	SFSF	GFSF	BFSF
Ash	15.50 ± 0.29^{c}	6.25 ± 0.14^{a}	5.25 ± 0.14^{a}	5.75 ± 0.58^{a}	6.70 ± 0.58^{b}
Moisture	8.47 ± 0.58^{c}	$1.05\pm.0.03^{b}$	0.75 ± 0.14^{a}	0.60 ± 0.05^{a}	0.70 ± 0.12^{a}
Fat	15.00 ± 0.58^{d}	10.5 ± 0.58^{a}	12.75 ± 0.14^{b}	12.75 ± 0.58^{b}	14.17 ± 0.65^{c}
Protein	15.01 ± 0.01^{a}	15.11 ± 0.05^{a}	15.03 ± 0.39^{a}	15.05 ± 0.04^{a}	15.11 ± 0.05^{a}
Carbohydrates	43.03 ± 1.44^{a}	66.13 ± 0.7^{c}	64.12 ± 0.72^{bc}	63.83 ± 0.07^{bc}	61.6 ± 1.31^{b}
Fibre	3.00 ± 0.32^{c}	1.07 ± 0.22^{a}	2.08 ± 0.21^{b}	2.03 ± 0.12^{b}	1.80 ± 0.06^{b}
Energy (Kcal)	379.13 ± 0.6^{a}	421.20 ± 2.1^{b}	439.75 ± 0.43^{c}	438.33 ± 4.97^{c}	441.22 ± 0.51^{c}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
SFSF- Fluted Pumpkin Soaked Fluted Pumpkin Seed Flour
GFSF- Germinated Fluted Pumpkin Seed Flour BFSF-
Boiled Fluted Pumpkin Seed Flour
UFSF- Unprocessed Fluted Pumpkin Seed Flour

4.1.5 Weekly weight changes of animals fed with experimental diets formulated with flours of unprocessed and processed *Citrullus lanatus*, *Moringa oleifera* and *Telfaira occidentalis* seeds

The weekly weight changes by animals fed with the experimental diets formulated with processed and unprocessed seed flours of *Citrullus lanatus, Moringa oleifera* and *Telfaira occidentalis* respectively and the control diet (containing groundnut cake) are represented in Figures 4.3a, 4.3b and 4.3c respectively. There was weight loss at the first week of feeding the animals with experimental diets in all the groups except for the control diet but gradual increase in the weight of the experimental animals in the subsequent weeks was noticed. Amongst animals fed with various experimental diets, those fed with soaked *Citrullus lanatus* seed flour supplemented diets had the highest weight gain at week four while unprocessed *Moringa oleifera* seed flour supplemented diet had the least weight gain at week four.

On a general note, weight gain was observed in the entire experimental animal fed with the experimental diets.

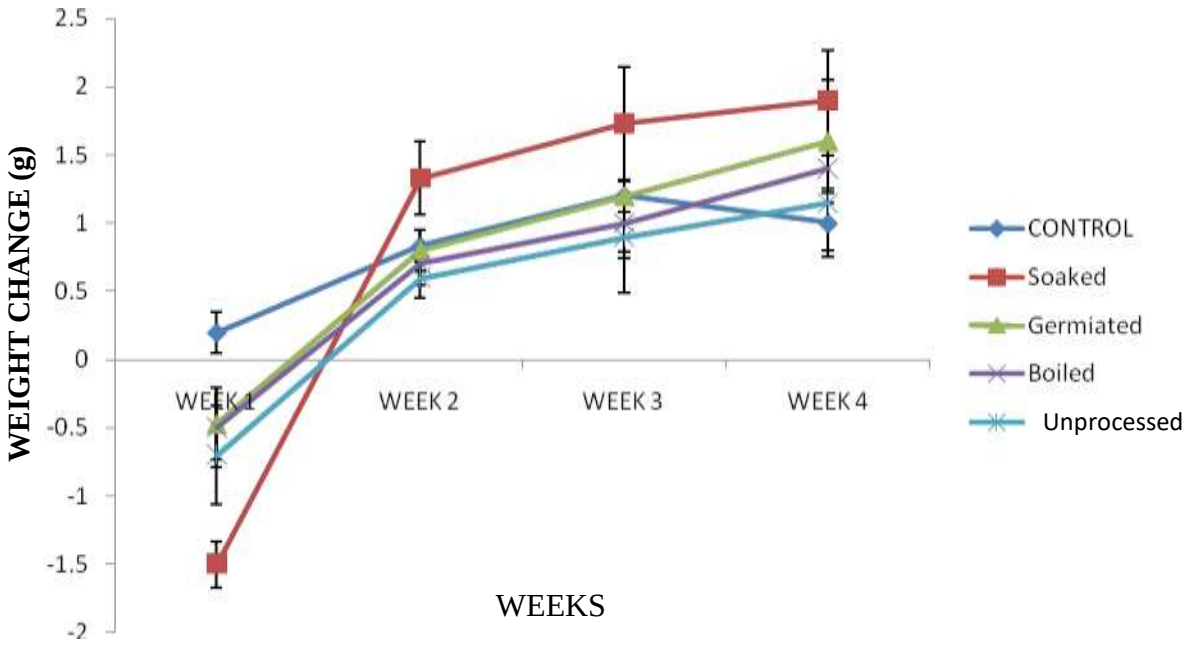


Figure 4.3a: Weekly Weight Changes of Rats Fed with *Citrullus lanatus* Seed Flour Supplemented Diets

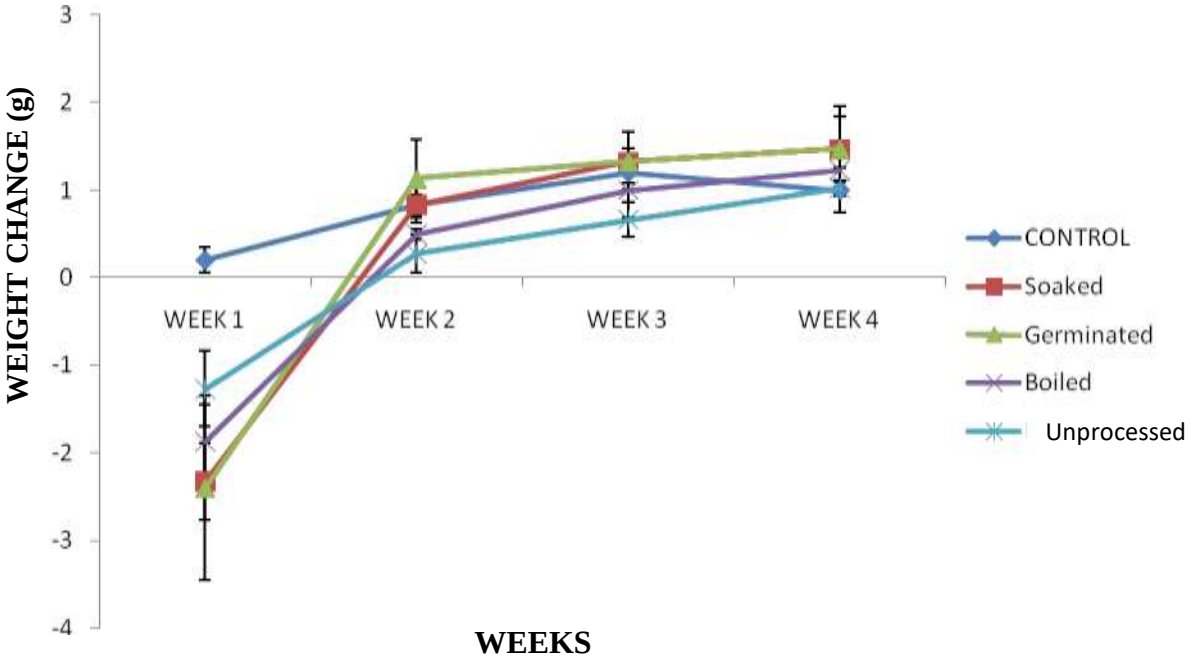


Figure 4.3b: Weekly Weight Changes of Rats Fed with *Moringa oleifera* Seed Flour Supplemented Diets

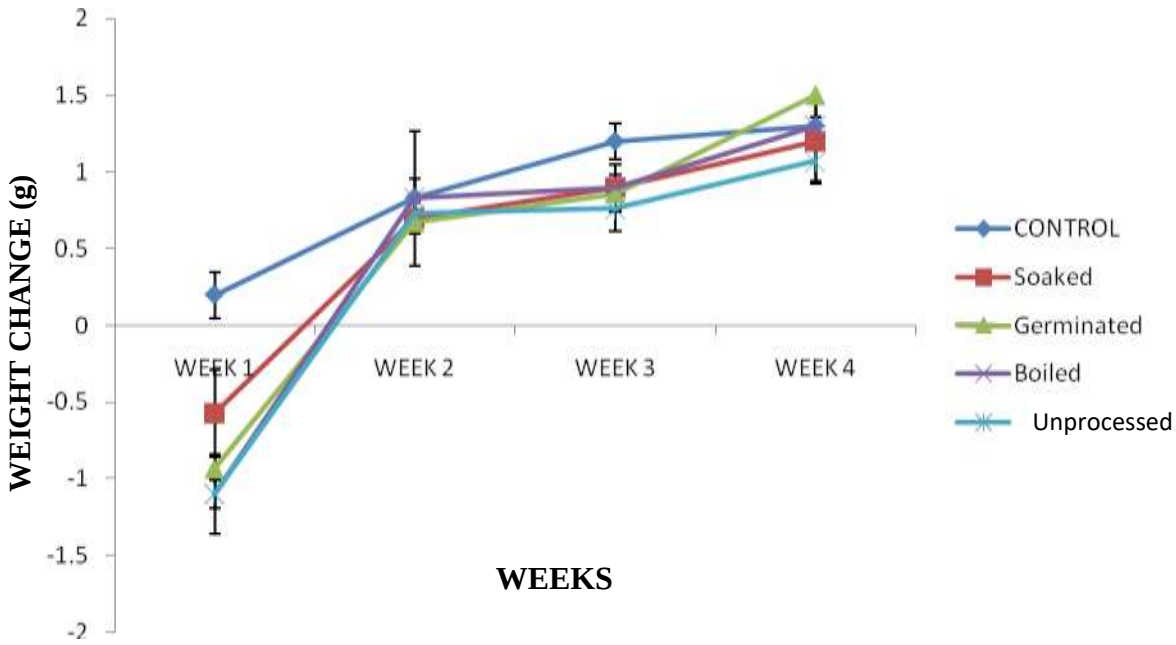


Figure 4.3c: Weekly Weight Changes of Rats Fed with *Telfaira occidentalis* Seed Flour Supplemented Diets

4.1.6 Biochemical parameters of animals fed with supplemented diets of unprocessed and processed seed flours of *Citrullus lanatus, Moringa oleifera* and *Telfaira occidentalis*

The results for liver and renal function test of animals fed with supplemented diets of unprocessed and processed seed flours of *Citrullus lanatus, Moringa oleifera* and *Telfaira occidentalis* are reported below.

4.1.6.1 *Liver and renal function indices of animals fed with supplemented diets of unprocessed and processed seed flours of Citrullus lanatus, Moringa oleifera Lam. and Telfaira occidentalis*

The biochemical parameters of animals fed with supplemented diets of unprocessed and processed seed flours of watermelon, moringa and fluted pumpkin for 28 days are shown in Tables 4.8a, 4.8b and 4.8c respectively.

The processing treatment significantly ($p < 0.05$) reduced serum AST, ALT and ALP activities of animals fed with processed seed flour of watermelon supplemented diet when compared to the unprocessed (Table 4.8a). The serum creatinine level of the animals on control diet was significantly ($p < 0.05$) higher when compared to animals placed on supplemented watermelon seed flour diets. There was no significant ($p > 0.05$) difference in serum sodium level of animals fed soaked watermelon seed flour supplemented diet when compared to animals on control diet but a significant ($p < 0.05$) difference was observed when compared to animals in other watermelon supplemented diets.

Significant ($p<0.05$) decrease was observed in serum ALT and AST and ALP in all processed seed flour of moringa except in boiled moringa supplemented diet where ALP was significantly ($p<0.05$) higher when compared to that of unprocessed seed flour as presented in Table 4.8b. All moringa seed flour showed significant ($p<0.05$) decrease in creatinine and sodium levels when compare to the control. No significant ($p>0.05$)

difference observed in chloride level of unprocessed and germinated moringa seed flour supplemented diets.

The processing methods also revealed significantly ($p < 0.05$) reduction in serum AST, ALT and ALP activities of animals fed with processed seed flour of fluted pumpkin supplemented diets (Table 4.8c). There was significant($p < 0.05$) difference in the serum bilirubin and total protein levels of animals on fluted pumpkin diets, significant ($p < 0.05$) difference in the serum albumin levels of animals fed with different supplemented diets was noticed.

The potassium level of animals fed with germinated *Telfaira occidentalis* seed flour supplemented diet was significantly ($p < 0.05$) higher when compared to those of others in group. There was significant ($p < 0.05$) difference in the serum urea level of animal fed seed flour supplemented diets of *Telfaira occidentalis* when compared to the control diet.

Table 4.8a: Biochemical Parameters of Animals Fed with Supplemented Diets of Unprocessed and Processed *Citrullus Lanatus* (Watermelon) Seed Flours

BIOCHEMICAL PARAMETERS	CONTROL	UWMSF	SWMSF	GWMSF	BWMSF
AST (U/L)	210.70±2.14^{a}	320.1±4.69^{e}	304.4±5.27^{b}	313.8± 3.41^{c}	318.5±6.34^{d}
ALT (U/L)	60.6±0.30^{a}	179.9±2.44^{e}	166.1±2.91^{d}	125.0± 3.82^{b}	150.8±2.13^{c}
ALP (U/L)	221.7±1.21^{b}	505.6± 3.43^{e}	229.7±1.38^{c}	259.3±2.05^{d}	176.4±0.95^{a}
TP(g/dL)	6.4±0.15^{a}	8.40±0.03^{c}	6.6±0.23^{a}	8.30±0.09^{c}	7.30 ±0.31^{b}
Albumin (g/dL)	3.0±0.01^{a}	3.3±0.02^{a}	3 .0±0.01^{a}	3.0±0.02^{a}	3.0±0.01^{a}
TB (mg/dL)	17.7±1.15^{b}	21.4±1.29^{c}	13.6±1.11^{a}	19.2±1.26^{b}	17.0±2.31^{b}
Urea (mg/dl)	8.3±0.80^{c}	12.4±0.72^{e}	7.8±1.01^{a}	9.3±0.47^{d}	8.10±0.99^{b}
Sodium(mmol/L)	130.7±4.2^{d}	118.9±2.62^{b}	131.3±5.10^{d}	128.0± 3.72^{c}	103.3± 4.21^{a}
Potassium(mmol/L)	6.4±0.24^{c}	9.6±0.40^{e}	6.0±1.01^{b}	5.9±0.42^{a}	6.7±0.72^{d}
Chloride(mmol/L)	98.0±1.12^{a}	107.2±1.51^{b}	97.0± 1.21^{a}	99.0±1.27^{a}	99.1±0.98^{a}
Creatinine(mg/dl)	0.9±0.01^{d}	0.5±0.01^{c}	0.30±0.02^{a}	0.40±0.02^{b}	0.40±0.01^{b}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)
TB –Total protein, TB- Total bilirubin, CB- Conjugated bilirubin
SWMSF –Soaked Watermelon Seed Flour
GWMSF- Germinated Watermelon Seed Flour
BWMSF- Boiled Watermelon Seed Flour
UWMSF- Unprocessed Watermelon Seed Flour

Table 4.8b: Biochemical Parameters of Animals Fed with Supplemented Diets of Unprocessed and Processed *Moringa oleifera* (Moringa) Seed Flours

BIOCHEMICAL PARAMETERS	CONTROL	UMSF	SMSF	GMSF	BMSF
AST (U/L)	210.70±2.14^{a}	244.5±4.75^{e}	222.0±2.22^{c}	232.4± 3.48^{d}	215.8±4.82^{b}
ALT (U/L)	60.6±0.30^{d}	67.3±1.52^{e}	57.2±1.51^{b}	59.5± 3.33^{c}	48.4±1.60^{a}
ALP (U/L)	221.7±1.21^{d}	186.7± 4.72^{c}	137.7±3.03^{a}	167.6±4.20^{b}	225.6±3.04^{e}
TP(g/dL)	6.4±0.15^{b}	7.20±0.05^{d}	5.8±0.11^{a}	7.10±0.05^{c}	6.50 ±1.12^{b}
Albumin (g/dL)	3.0±0.01^{b}	2.5±0.01^{a}	3 .1±0.01^{b}	3.0±0.01^{b}	3.2±0.01^{c}
T B (mg/dL)	17.7±1.15^{c}	18.0±0.04^{d}	10.1±1.51^{a}	16.6±1.32^{c}	14.4±2.04^{b}
Urea (mg/dl)	8.3±0.80^{a}	13.9±0.35^{e}	11.9±1.01^{d}	10.2±1.09^{b}	10.8±0.17^{c}
Sodium(mmol/L)	130.7±4.2^{d}	114.8±1.91^{b}	126.9±2.76^{c}	96.8± 3.65^{a}	112.0± 2.12^{b}
Potassium(mmol/L)	6.4±0.24^{b}	7.3±0.94^{d}	6.7±0.15^{c}	5.5±0.71^{a}	6.23±1.02^{b}
Chloride(mmol/L)	98.0±1.12^{a}	100.1±1.19^{c}	96.8± 1.40^{b}	100.6±1.01^{c}	98.5±1.18^{b}
Creatinine(mg/dl)	0.9±0.01^{d}	0.4±0.02^{c}	0.30±0.03^{b}	0.40±0.02^{c}	0.20±0.03^{a}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p < 0.05$)
TB –Total protein, TB- Total bilirubin, CB- Conjugated bilirubin
SMSF- Soaked Moringa seed flour
GMSF- Germinated Moringa seed flour
BMSF- Boiled Moringa seed flour
UMSF-Unprocessed Moringa seed flour

Table 4.8c: Biochemical Parameters of Animals Fed with Supplemented Diets of Unprocessed and Processed *Telfaira occidentalis* (Fluted Pumpkin) Seed Flour

BIOCHEMICAL PARAMETERS	CONTROL	UFSF	SFSF	GFSF	BFSF
AST (U/L)	210.70±2.14^{b}	244.5±2.15^{e}	217.5±5.39^{c}	239.3± 3.94^{d}	206.7±4.36^{a}
ALT (U/L)	60.6±0.30^{a}	118.3±2.32^{e}	114.4±2.80^{d}	106.3± 3.53^{c}	92.3±2.42^{b}
ALP (U/L)	221.7±1.21^{a}	328.4± 3.31^{e}	308.9±3.17^{d}	298.7±2.20^{c}	276.7±2.53^{b}
TP(g/dL)	6.4±0.15^{b}	6.0±0.06^{a}	7.4±0.16^{e}	7.10±0.23^{d}	6.80 ±0.55^{c}
Albumin (g/dL)	3.0±0.01^{b}	3.1±0.01^{c}	2.6±0.02^{a}	3.0±0.01^{b}	3.1±0.01^{c}
T B (mg/dL)	17.7±1.15^{a}	48.6±1.04^{d}	22.6±2.43^{b}	28.5±1.98^{c}	21.7±2.11^{b}
Urea (mg/dl)	8.3±0.80^{a}	11.4.±1.05^{e}	9.4±1.01^{c}	10.7±1.09^{d}	8.7±1.02^{b}
Sodium(mmol/L)	130.7±4.2^{e}	118.7±2.31^{d}	87.6±2.38^{b}	46.6± 2.75^{a}	112.7± 3.15^{c}
Potassium(mmol/L)	6.4±0.24^{a}	12.9±1.04^{d}	10.1±0.15^{c}	16.8±1.09^{e}	9.0±0.35^{b}
Chloride(mmol/L)	98.0±1.12^{b}	93.9±2.12^{a}	101.4± 1.60^{c}	97.2±2.10^{b}	106.4±3.05^{d}
Creatinine(mg/dl)	0.9±0.01^{d}	0.6±0.02^{c}	0.40±0.02^{a}	0.40±0.01^{a}	0.50±0.03^{b}

Values are expressed as means of triplicate determinations ± SEM.Values along rows with different superscript are significantly different ($p<0.05$)
TB –Total protein, TB- Total bilirubin, CB- Conjugated bilirubin
SFSF- Soaked Fluted Pumpkin Seed Flour
GFSF- Germinated Fluted Pumpkin Seed Flour
BFSF- Boiled Fluted Pumpkin Seed Flour
UFSF- Unprocessed Fluted Pumpkin Seed Flour

4.1.6.2 *The serum lipid profile of animals fed with supplemented diets of processed and unprocessed seed flours of Citrullus lanatus, Moringa oleifera and Telfaira occidentalis*

The serum lipid profile parameters of animals fed with supplemented diets of processed and unprocessed seed flours of watermelon, moringa and fluted pumpkin for 28 days are shown in Figure 4.4a, 4.4b and 4.4c respectively.

The serum cholesterol and triglyceride levels of animals fed with soaked, germinated and boiled watermelon seed flour supplemented diets were significantly ($p < 0.05$) higher when compared with their control but significantly ($p<0.05$) lower when compared with their unprocessed seed flour group (Figures 4.4a). There was significant ($p<0.05$) difference in the HDL and LDL levels of animals fed with processed supplemented diets when compared with the control and unprocessed groups.

There was significant ($p<0.05$) difference seen in cholesterol, triglyceride, LDL and HDL levels in all the groups fed processed and unprocessed moringa supplemented diets as shown in Figure 4.4b.

Significant ($p<0.05$) decrease was seen in cholesterol and LDL levels of animals fed processed fluted pumpkin when compared to animals fed unprocessed fluted pumpkin (Figure 4.4c).

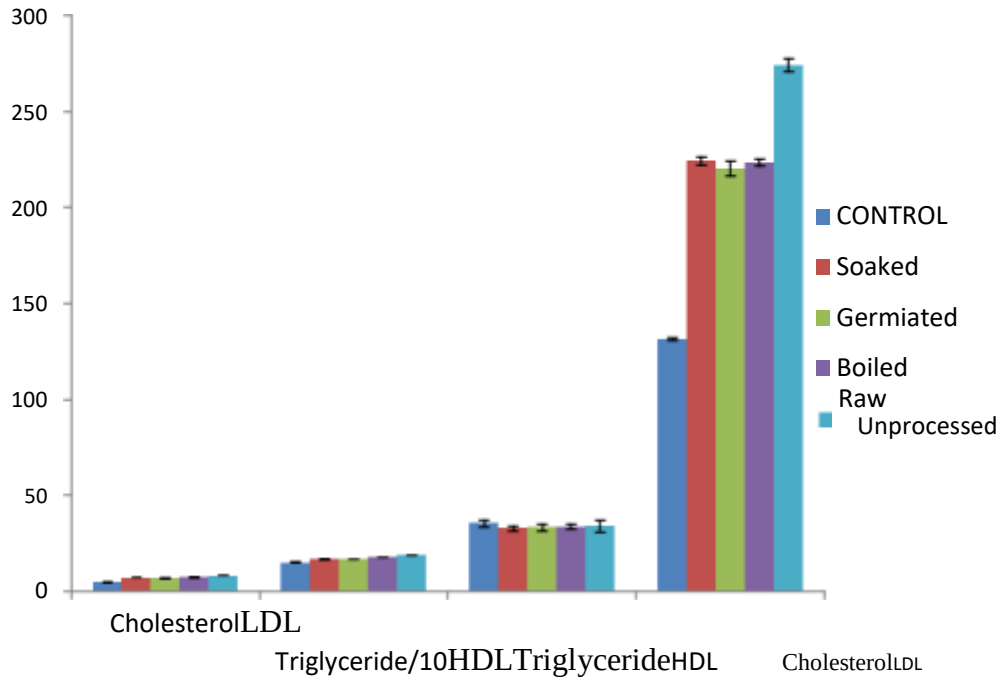


Figure 4.4a: Serum Lipid Profile Parameters of Rats Fed with Processed and Unprocessed *Citrullus Lanatus* Seed Flour Supplemented Diets

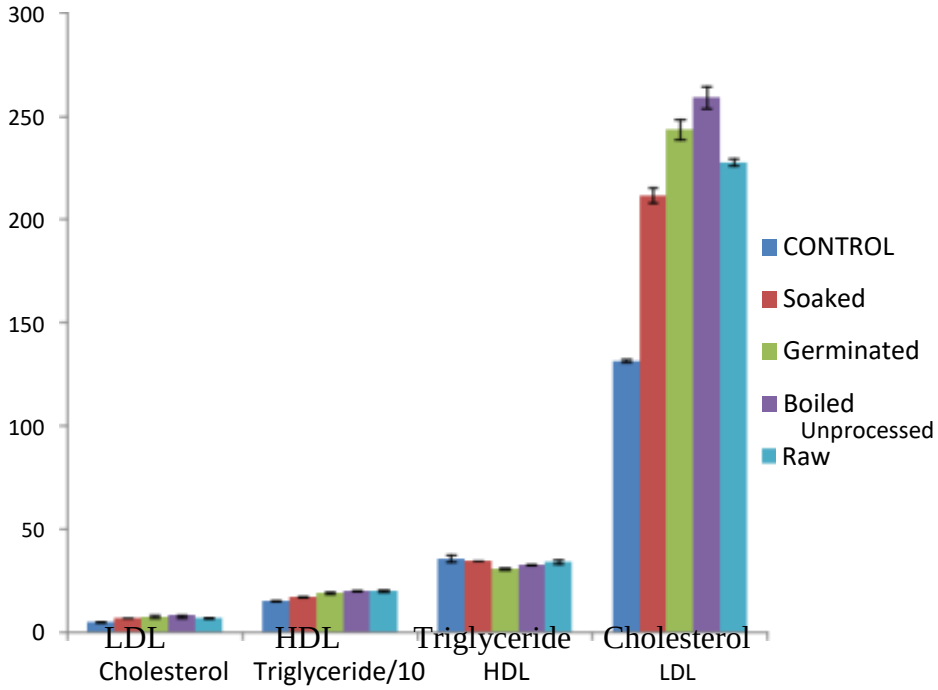


Figure 4.4b: Serum Lipid Profile Parameters of Rats Fed with Processed and Unprocessed *Moringa oleifera* Seed Flour Supplemented Diets

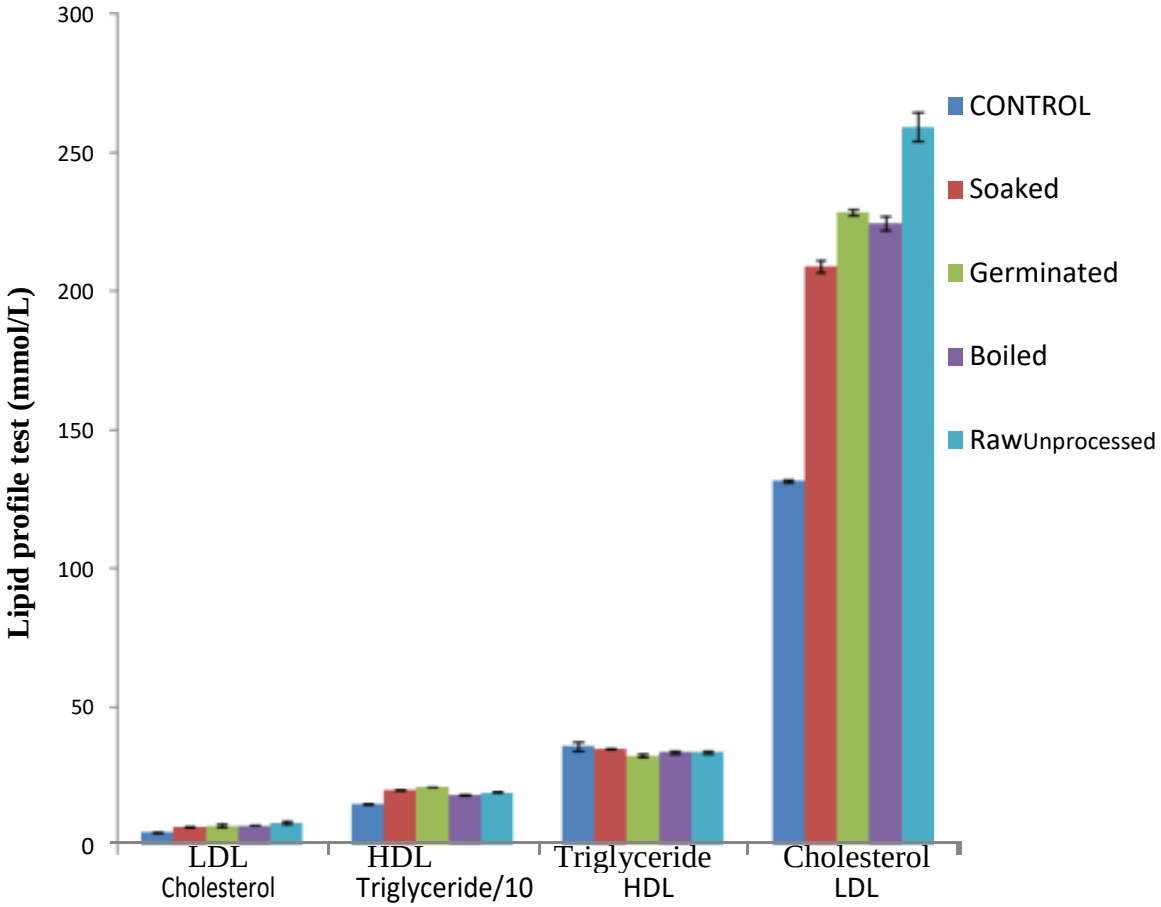


Figure 4.4c: Serum Lipid Profile Parameters of Rats Fed with Processed and Unprocessed *Telfaira occidentalis* Seed Flour Supplemented Diets

4.1.7 Haematological parametersof rats fed with supplemented diets of unprocessed and processed seed flours of *Citrullus lanatus, Moringa oleifera* and *Telfaira occidentalis*

Haematological parameters of animals fed with supplemented diets of processed and unprocessed seed flours of watermelon, moringa and fluted pumpkin supplemented diets respectively for 28 days are represented in Tables 4.9a, 4.9b and 4.9c.

The PCV, haemoglobin (HB) and RBC levels of animal fed with the watermelon seeds supplemented diets were significantly ($p < 0.05$) higher when compared to animals on its unprocessed supplemented diet (Table 4.9a). Unprocessed watermelon seed flour supplemented diet led to a significant ($p < 0.05$) increase in the WBC of animals as compared to animals fed with processed watermelon experimental diets. The neutrophil, lymphocyte, monocyte, basophil and eosinophil counts in animals on control diet were significantly ($p < 0.05$) difference when compared to animals placed on other supplemented diets.

The PCV, WBC, platelet, RBC and haemoglobin levels of animals fed with processed moringa seed flour supplemented diets were significantly ($P<0.05$) difference to those placed on control diets and unprocessed seed flour (Table 4.9b). Eosinophil, neutrophil, basophil and monocyte counts was significantly ($p<0.05$) higher in animal fed germinated moringa seed flour supplemented diet.

RBC was significantly ($p<0.05$) higher in rats fed soaked and germinated fluted pumpkin seed flour supplemented diets when compared to rats fed unprocessed fluted pumpkin seed flour supplemented diet (Table 4.9c), haemoglobin level was significantly higher in rats fed soaked and boiled fluted pumpkin seed flour supplemented diet when compared to animal fed unprocessed fluted pumpkin seed flour supplemented diet.RBC, platelet and lymphocyte levels were higher in rats fed soaked fluted pumpkin seed flour

supplemented diet while eosinophil, neutrophil, basophil and monocyte counts were significantly ($p<0.05$) difference in rats fed processed fluted pumpkin seed flour supplemented diets when compare to rats fed on it unprocessed diet.

Table 4.9a: Haematological Parameters of Rats Fed Unprocessed and Processed *Citrullus lanatus* Supplemented Diets

HAEMATOLOGICAL PARAMETERS	CONTROL	UWMSF	SWMSF	GWMSF	BWMSF
HB (g/dl)	16.2 ± 0.15^{e}	9.9 ± 0.5^{a}	10.3 ± 0.33^{b}	11.8 ± 0.08^{c}	12.1 ± 0.4^{d}
PCV (%)	48.0 ± 1.2^{d}	29.0 ± 0.8^{a}	31.0 ± 0.4^{b}	35.0 ± 1.0^{c}	36.0 ± 0.9^{c}
MCV (Fl)	87.0 ± 0.04^{d}	77.0 ± 0.01^{c}	76.0 ± 0.03^{b}	71.0 ± 0.13^{a}	7.01 ± 0.1^{a}
MCH (Pg)	22.0 ± 0.23^{b}	22.0 ± 0.18^{b}	20.0 ± 0.81^{a}	24.0 ± 0.62^{c}	24.0 ± 0.59^{c}
MCHC (g/dl)	33.0 ± 0.56^{b}	31.0 ± 0.22^{a}	$33.0\pm.55^{b}$	34.0 ± 0.72^{c}	$34.0\pm.70^{c}$
RBC($\times10^{9}$/l)	4.9 ± 0.03^{c}	4.5 ± 0.12^{a}	4.7 ± 0.04^{b}	4.9 ± 0.05^{c}	5.1 ± 0.01^{d}
WBC ($\times10^{9}$/l)	5.2 ± 0.31^{a}	7.9 ± 0.21^{e}	5.8 ± 0.29^{b}	6.7 ± 0.1^{d}	6.4 ± 0.09^{c}
PLC($\times10^{9}$/l)	278.0 ± 45.21^{e}	267.0 ± 15.86^{d}	223.0 ± 20.05^{a}	249.0 ± 11.3^{b}	261.0 ± 35.34^{c}
L	58.0 ± 5.25^{c}	49.0 ± 2.98^{b}	60.0 ± 3.91^{c}	38.0 ± 3.21^{a}	38.0 ± 4.0^{a}
NEU	42.0 ± 6.13^{b}	33.0 ± 0.18^{a}	34.0 ± 0.20^{a}	52.0 ± 6.87^{c}	59.0 ± 7.52^{d}
BASO	1.0 ± 0.17^{b}	3.0 ± 0.09^{c}	00.0 ± 0.00^{a}	1.0 ± 0.11^{b}	00.0 ± 0.00^{a}
ECO	3.0 ± 0.07^{b}	5.0 ± 0.3^{c}	3.0 ± 0.09^{b}	3.0 ± 0.11^{b}	2.0 ± 0.02^{a}
MONO	6.0 ± 0.09^{c}	10.0 ± 0.11^{d}	3.0 ± 0.04^{b}	6.0 ± 0.08^{c}	1.0 ± 0.02^{a}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)

Pack Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Lymphocytes (L), Monocytes (MONO), Eosinophils (ECO), Neutrophils (NEU), Basophils (BASO) and Haemoglobin Concentration (Hb)

SWMSF –Soaked Watermelon Seed Flour

GWMSF- Germinated Watermelon Seed Flour

BWMSF- Boiled Watermelon Seed Flour

UWMSF- Unprocessed Watermelon Seed Flour

Table 4.9b: Haematological Parameters of Rats Fed Unprocessed and Processed *Moringa oleifera* Supplemented Diets

Haematological Parameters	CONTROL	UMSF	SMSF	GMSF	BMSF
HB (g/dl)	16.2±0.15^{d}	10.8±0.09^{b}	11.3±0.13^{c}	9.0±0.03^{a}	10.7±0.05^{b}
PCV (%)	48.0±1.2^{e}	35.0±1.51^{d}	33.0±1.31^{c}	27.0±1.02^{a}	32.0±1.29^{b}
MCV (Fl)	87.0±0.04^{e}	79.0±1.05^{d}	74.0±0.05^{b}	73.0±0.06^{a}	77.0±1.01^{c}
MCH (Pg)	22.0±0.23^{b}	26.0±0.29^{c}	22.0±0.25^{b}	21.0±0.21^{a}	22.0±0.24^{b}
MCHC (g/dl)	33.0±0.56^{b}	32.0±1.01^{c}	29.0±1.03^{a}	33.0±1.15^{d}	30.0±1.00^{a}
RBC(×10^{9}/l)	4.9±0.03^{b}	6.3±0.15^{d}	5.7±0.12^{c}	4.3±0.09^{a}	4.8±0.12^{b}
WBC (×10^{9}/l)	5.2±0.31^{a}	5.8±0.51^{b}	10.9±0.21^{c}	13.5±0.31^{d}	11.5±0.11^{c}
PLC(×10^{9}/l)	278.0±45.21^{e}	251.0±41.21^{c}	253.0±35.05^{d}	247.0±23.15^{b}	189.0±13.02^{a}
L	58.0±5.25^{c}	72.0±4.01^{d}	34.0± 3.11^{b}	28.0± 1.51^{a}	81.0±5.21^{e}
NEU	42.0±6.13^{c}	25.0± 3.41^{b}	63.0± 6.05^{e}	60.0±5.55^{d}	12.0±2.45^{a}
BASO	1.0±0.17^{b}	0.0±0.00^{a}	0.0±0.00^{a}	2.0±0.05^{c}	1.0±0.01^{b}
ECO	3.0±0.07^{b}	1.0±0.02^{a}	1.0±0.02^{a}	3.0±0.04^{b}	1.0±0.01^{a}
MONO	6.0±0.09^{c}	2.0±0.02^{a}	2.0±0.01^{a}	7.0± 0.08^{d}	5.0±0.04^{b}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p<0.05$)

Pack Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Lymphocytes (L), Monocytes (MONO), Eosinophils (ECO), Neutrophils (NEU), Basophils (BASO) and Haemoglobin Concentration (Hb)

SMSF- Soaked Moringa seed flour

GMSF- Germinated Moringa seed flour

BMSF- Boiled Moringa seed flour

UMSF-Unprocessed Moringa seed flour

Table 4.9c: Haematological Parameters of Rats Fed Unprocessed and Processed *Telfaira occidentalis* Supplemented Diets

Haematological Parameters	CONTROL	UFSF	SFSF	GFSF	BFSF
HB (g/dl)	16.2±0.15^{d}	11.7±0.09^{b}	12.1±0.15^{c}	10.5±0.07^{a}	12.0±0.13^{c}
PCV (%)	48.0±1.2^{e}	35.0±1.15^{b}	36±1.25^{c}	34.0±1.03^{a}	36.0±1.58^{d}
MCV (Fl)	87.0±0.04^{e}	70.0±0.01^{a}	74.0±0.06^{d}	73.0±0.04^{c}	71.0±0.02^{b}
MCH (Pg)	22.0±0.23^{a}	24.0±0.23^{b}	25.0±0.31^{c}	24.0±0.24^{b}	24.0±0.22^{b}
MCHC (g/dl)	33.0±0.56^{b}	31.0±1.11^{a}	32.0±1.08^{a}	33.0±1.12^{b}	33.0±1.09^{b}
RBC(×10^{9}/l)	4.9±0.03^{a}	4.9±0.07^{a}	6.0±0.09^{c}	6.4±0.13^{c}	5.1±0.11^{b}
WBC (×10^{9}/l)	5.2±0.31^{d}	4.2±0.15^{b}	3.7±0.11^{a}	4.7±0.13^{c}	6.6±0.17^{e}
PLC(×10^{9}/l)	278.0±45.21^{e}	201.0±28.16^{b}	265.0±35.57^{d}	184.0±24.72^{a}	233.0±30.21^{c}
L	58.0±5.25^{c}	63.0±6.02^{d}	78.0± 5.24^{e}	47.0± 4.58^{b}	28.0± .21^{a}
NEU	42.0±6.13^{d}	31.0±4.92^{b}	19.0± 2.21^{a}	35.0±5.24^{c}	52.0±7.54^{e}
BASO	1.0±0.17^{c}	1.0±0.01^{b}	0.0±0.00^{a}	2.0±0.04^{d}	1.0±0.02^{b}
ECO	3.0±0.07^{c}	2.0±0.02^{b}	1.0±0.02^{a}	3.0±0.05^{c}	4.0±0.04^{d}
MONO	6.0±0.09^{c}	3.0±0.03^{b}	2.0±0.01^{a}	13.0±1.21^{d}	15.0±1.54^{e}

Values are expressed as means of triplicate determinations ± SEM.Values along rows with different superscript are significantly different ($p<0.05$)

Pack Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Lymphocytes (L), Monocytes (MONO), Eosinophils (ECO), Neutrophils (NEU), Basophils (BASO) and Haemoglobin Concentration (Hb)

SFSF- Soaked Fluted Pumpkin Seed Flour

GFSF- Germinated Fluted Pumpkin Seed Flour

BFSF- Boiled Fluted Pumpkin Seed Flour

UFSF- Unprocessed Fluted Pumpkin Seed Flour

4.1.8 Histomicrographs of liver and kidney of animals fed with supplemented diets of unprocessed and processed seed flours of *Citrullus lanatus, Moringa oleifera Lam.* and *Telfaira occidentalis*

The histomicrographs of the liver, and kidney of rats fed watermelon moringa and fluted pumpkin seed flour supplemented diets are showsn in Plate I - VI.

Normal hepatocyte of animals fed boiled, soaked and germinated watermelon seed flour while a mild portal tract inflamation was seen in the hepatocyte of animals fed unprocessed watermelon seed flour supplemented diet group as revealed in Plate I.

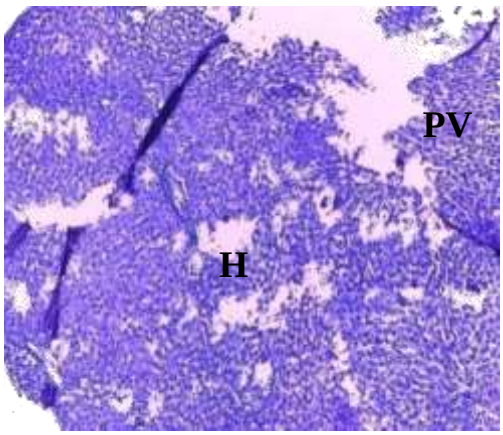
Normal architecture of glomerulus and tubules of all animals fed watermelon seed flour supplemented diet was shown in Plate II.

It is shown in Plate III, normal portal vein and hepatocyte of animal fed moringa seed flour supplemented diets

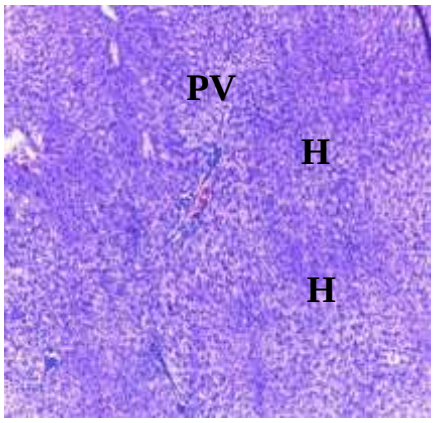
Normal traverse sections of kidney of rats fed moringa seed flour supplemented diets was revealed in Plate IV.

Normal hepatocyte of rat fed fluted pumpkin seed flour supplemented diets was seen in Plate V except for the group of animal fed unprocessed fluted pumpkin seed flour supplemented diets which showed confluence fenthery degeneration of the hepatocyte.

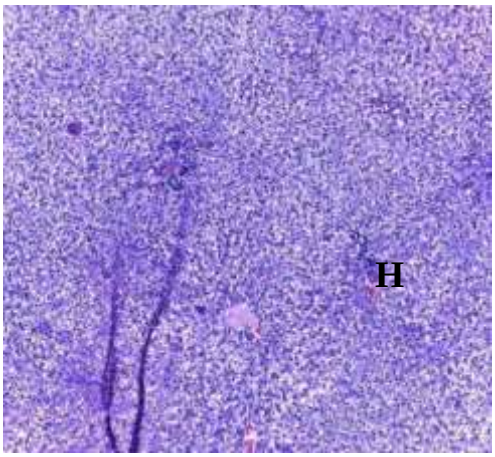
Normal traverse sections of kidney of rats fed fluted pumpkin seed flour supplemented diets was observed in Plate VI except for the group of animal fed unprocessed fluted pumpkin seed flour supplemented diets which showed moderate tubulo-intertitial inflammation.



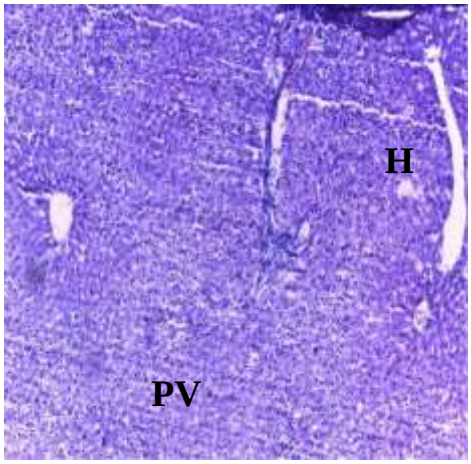
a) Liver hepatocyte of rats administered unprocessed watermelon seed flour showing mild portal tract inflamation



b) Soaked watermelon seed flour showing normal liver morphology

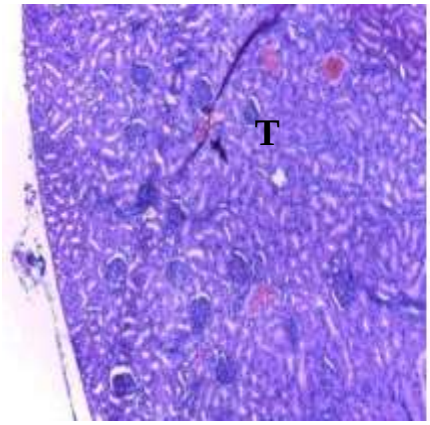


c) Germinated watermelon seed flour showing nomal morphology of liver

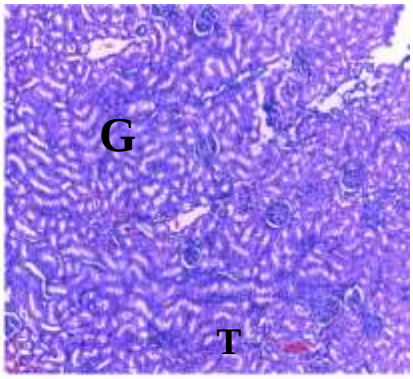


d) Boiled watermelon seed flour showing normal hepatocyte and absence of steatosis

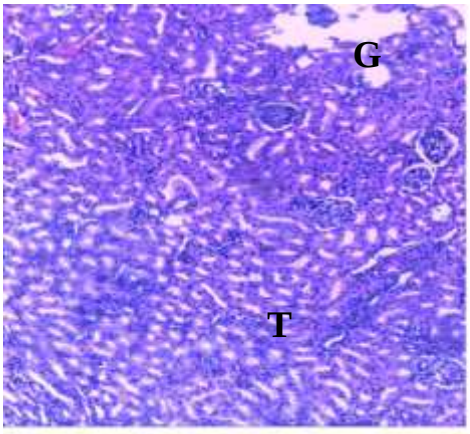
Plate I: Photomicrograph of Liver Sections of Rats Fed *Citrullus lanatus* seed flour Supplemented Diets Showing Normal Hepatocyte (H) and Portal Vein (PV) for b, c and d while mild inflammation for a



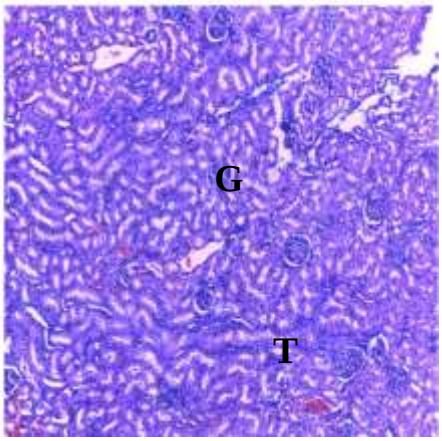
a) Unprocessed watermelon seed flour showing moderate tubulus



b) Boiled watermelon seed flour showing normal kidney architecture



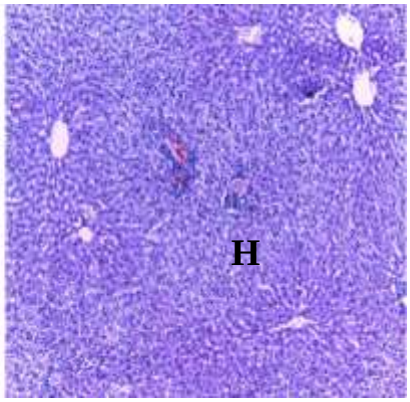
c) Germinated watermelon seed flour showing normal G and



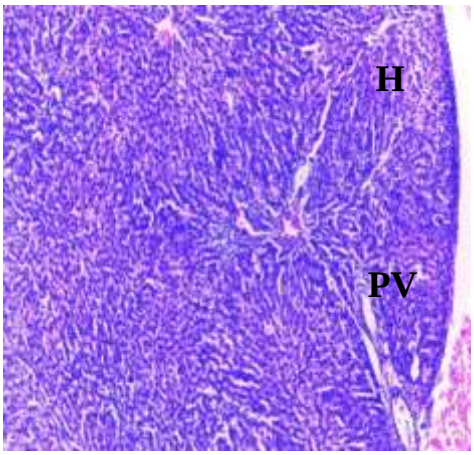
d) Boiled watermelon seed flour showing normal kidney morphology

Plate II: Photomicrograph of Kidney Sections of Rats Fed *Citrullus Lanatus* seed flour Supplemented Diets Showing Normal Architecture of Glomerulus (G) and Tubules (T)

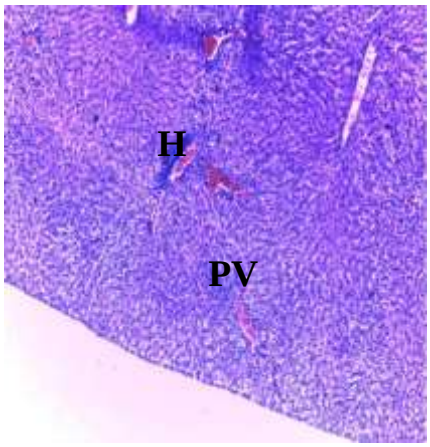
a) Unprocessed *Moringa* seed flour showing normal distribution of hepatocyte



b) Soaked *Moringa oleifera* seed flour showing a discrete increase in nucleus and cytoplasm relationship

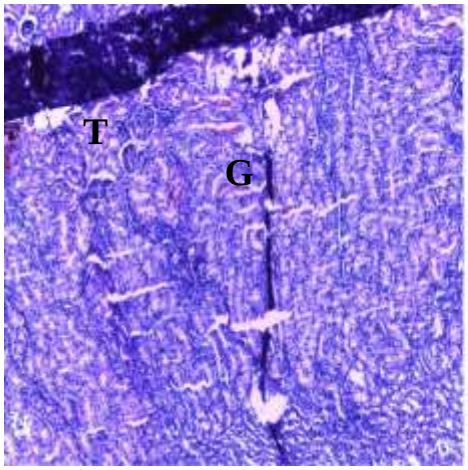


c) Germinated *Moringa* seed flour showing normal portal vein

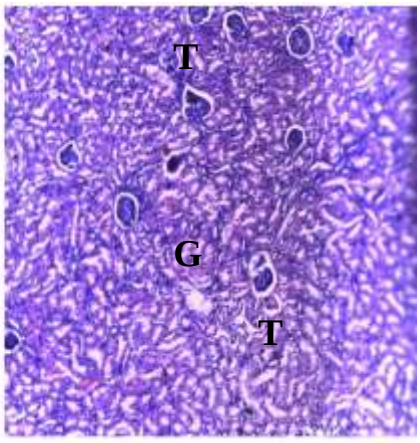


d) Boiled *Moringa* seed flour showing presence of macrophages and the absence of steatosis

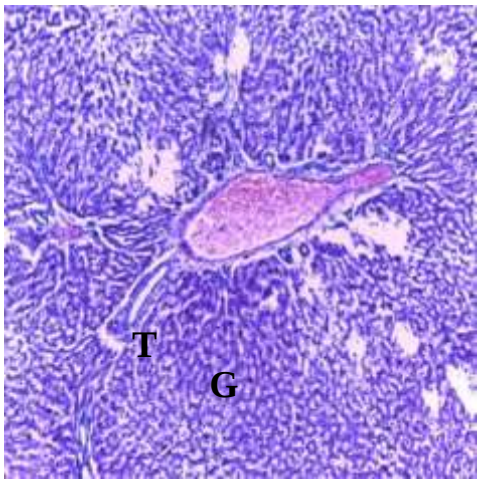
Plate III: Photomicrograph of Liver Sections of Rats Fed *Moringa oleifera* seed flour Supplemented Diets Showing Moderate Hepatocyte (H) and Portal Vein (PV)



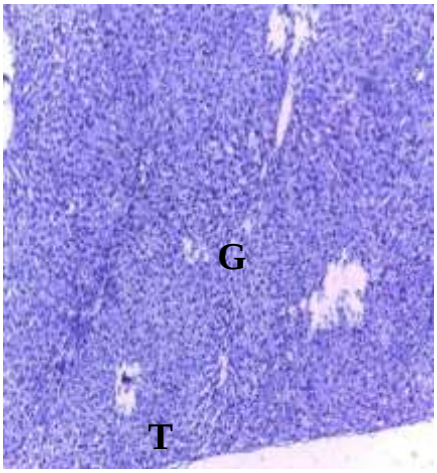
a) Unprocessed moringa seed flour showing normal tubules and glomerulus



b) Soaked moringa seed flour showing a normal kidney morphology

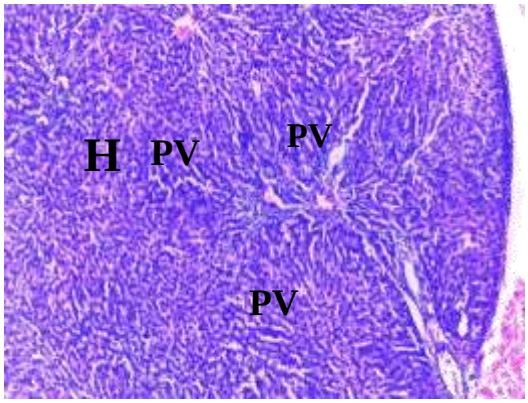


c) Germinated moringa seed flour showing normal glumerolus

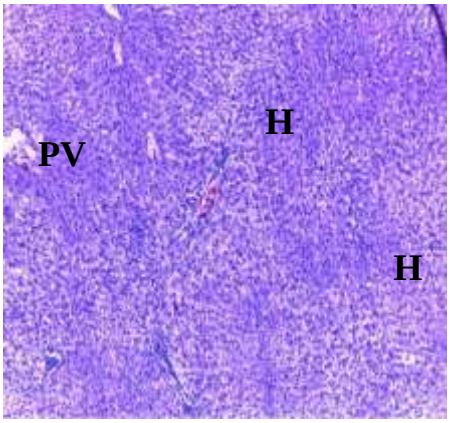


d) Boiled moringa seed flour showing normal tubules and glomerulus

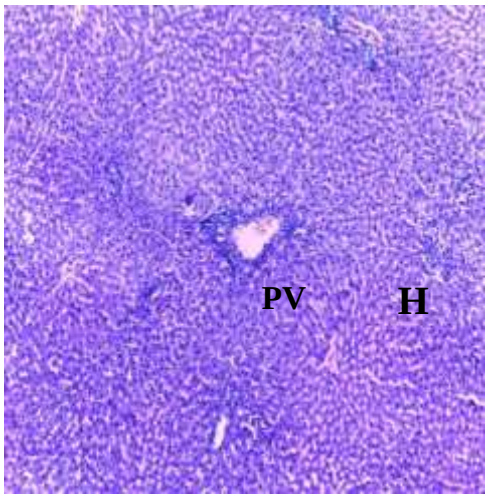
Plate IV: Photomicrograph of Kidney Sections of Rats Fed *Moringa oleifera* seed flour Supplemented Diets Showing Moderate Glomerulus (G) and Tubules (T)



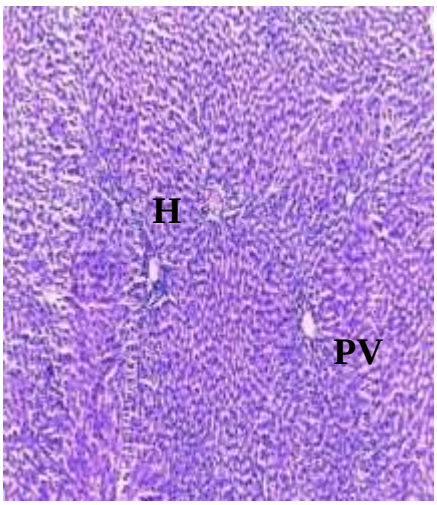
a) Unprocessed fluted pumpkin showing confluence fenthery degeneration of the hepatocyte.



b) Soaked fluted pumpkin seed flour showing normal portal vein

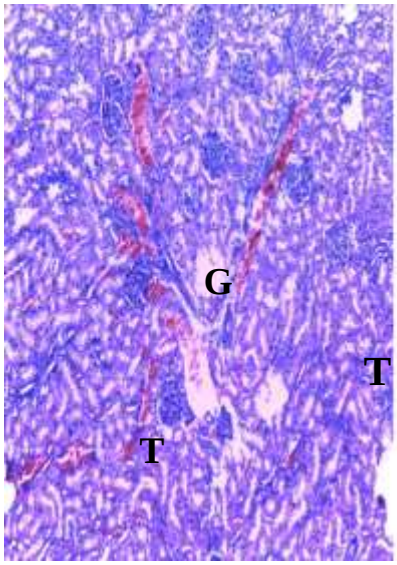


c) Germinated fluted pumpkin seed flour showing normal hepatocyte and portal vein

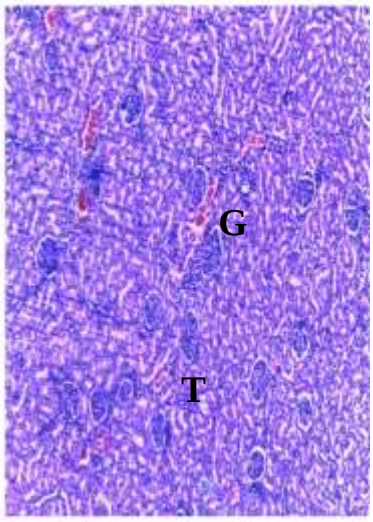


d) Boiled fluted pumpkin seed flour showing normal hepatocyte

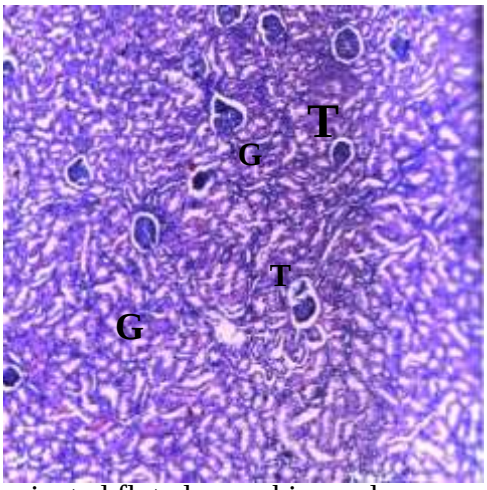
Plate V: Photomicrograph of Liver Sections of Rats Fed *Telfaira occidentalis* seed flour Supplemented Diets Showing Normal Hepatocyte (H) and Portal Vein (PV) for b, c and d While Confluence Fenthery Degeneration for a



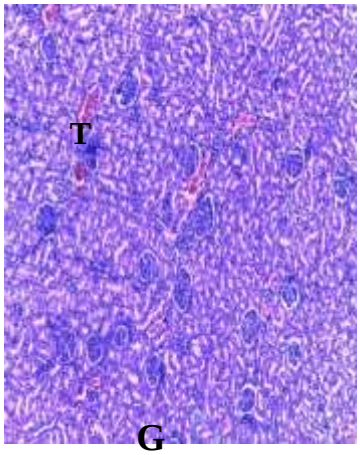
a) Unprocessed fluted pumpkin seed flour moderate tubulo-intertitial



b) Soaked fluted pumpkin seed flour showing normal tubule-interstitial morphology



c) Germinated fluted pumpkin seed flour showing moderate tubule-interstitial inflammation



d) Boiled fluted pumpkin seed flour showing moderate glumerulus

Plate VI: Photomicrograph of Kidney Sections of Rats Fed *Telfaira occidentalis* seed flour Supplemented Diets

4.2 Discussion

4.2.1 Proximate composition of unprocessed and processed watermelon, moringa and fluted pumpkin seeds

Moisture content is important in predicting the shelf life of food products, higher moisture content naturally encourage organisms present in flour to grow and cause deterioration. Result from this study (Tables 4.1a, 4.1b and 4.1c), showed significant decrease in the moisture contents of all the three seed flours in the processed state compared to the unprocessed state when compared. This could be due to the processing techniques which might have effect on the hydroscopic character of the seed flours which suggest decrease in the ability of the processed seed flour to interact with water. The lower moisture content of thee germinated seeds, might be as a result of water utilization for metabolic activities initiated by soaking. The lower moisture content obtained in this study suggest higher dry matter yield (Bamigboye *et al*., 2010) and ability to be kept for long periods without fear of deterioration or spoilage hence, lower moisture content also indicate reduction in microorganism activity in the seed flours which in turn will increase the presence and quality of nutrients in a food sample and these could enhance storage stability by increasing the shelf life of the seed flours (Nielsen, 2002; Olitino *et al*., 2007).

The current study indicated that, the processed seed flour of *Citrullus lanatus* showed steady increase in ash content when compared to its unprocessed seed flour and other seed flours *(Moringa oleifera* and *Telfaira occidentalis).* The ash content in this study (which ranges from 1.70 ± 0.15 to 4.93 ± 0.23) is lower than that of jackbean (6.51± 0.28) as reported by Olalekan and Bosede (2010). The low ash content in the seed flours might be as a result of leaching during steeping and washing.

The proximate composition showed that the three different seed flours (processed and unprocessed) are good sources of protein. Proteins are macronutrients which are known to be body building nutrients, they are essential components of cells and they perform various functions as enzymes, hormones, transport molecules, defense molecules (Immunoglobulins) and are involved in maintainance of osmotic and acid base balance (plasma proteins).The protein content ranges from 13.25 ± 1.70 in germinated *Citrullus lanatus* to 32.13 ± 2.33 in germinated *Moringa oleifera,* this is higher when compared to the protein content of some commonly consumed seeds in Nigeria, namely *coloccynthis citrullus,* rapeseed and sunflower (Adesuyi and Ipinmoroti, 2011), but lower when compared to that of groundnut cake 50 – 55 % (USDA, 2016).

Increased protein content seen in germinated *Moringa oleifera* and *Telfaira occidentalis* may be attributed to extensive breakdown of seed storage compound and synthesis of structural protein and other cell components occurring during germination. High amino acids biosynthetic activities in seedlings could result in increased content of free amino acids to support protein synthesis (Kuo *et al*., 2004), this report is similar to that of Rumiyati *et al*. (2012) who reported that germination increase the protein content of Australian sweet lupin *(Lupinus angustifolius L)*and is also similar to the findings of other researchers like (Nnam, 1995; Oshodi *et al*., 1999), who reported that protein level of germinated and fermented seeds increases when compared with unprocessed products.

The increased protein value of the processed seed flours could be attributed to the biochemical activities of the germinating seeds and the activities of the microorganisms during processing. Previous studies reported that during germination, carbohydrates are mobilized to synthesize amino acids for the growing seedling and proliferations of the microorganisms (Abdelrahaman *et al*., 2007; Dubey *et al*., 2008; Ochanda *et al*., 2010).

The protein contents of these processed seed flours are higher when compared to the protein content in egg yolk (14.56 %) (Dilawar *et al*., 2021) and Nigerian *P. elongatus* 18.2 % (Njinkoue *et al*., 2016). The high protein content in these processed seed flours (>15 %) showed that these seeds belong to the high protein seed category (WHO, 2007). The fibre content reduced significantly by soaking, germination and boiling in *Citrullus lanatus* and *Moringa oleifera,* these might be due to degradation of fibre into simpler sugar by initiated endogenous enzymes. This result is similar with previous studies on chickpea and kidney beans reported by Dejene (2015). However, fibre content of *Citrullus lanatus* and *Moringa oleifera* processed seed flours are still higher than of avocado (4.91%) which is considered to be rich in fibre, so therefore the seed flours in this research work can be consider as good sources of fibre and could be essential for normal excretion of waste materials in human and animal system.

Fat content in the three seed flours were higher in soaking (might be due to fermentation) and boiling but lower in germination, this decreased might be attributed to its usage as energy source to kick start germination (Beruk, 2015).

The carbohydrate value of all the seed flours ranges from 17.11±0.70 to 58.23±1.55, and these were higher when compared to carbohydrate from some animal foods (Njinkoue *et al*., 2016). Energy value of all the seed flours ranges from 490.38 Kcal/100g ±0.22 to 669.82 Kcal/100 g ± 0.67, higher energy value was seen in all the soaked and boiled seed flours while lower energy value was seen in all germinated seed flours when compared to their respective unprocessed seed flours. The energy value of any food sample is a function of its protein fat and carbohydrate contents.

4.2.2 Mineral composition of unprocessed and processed watermelon, moringa and fluted pumpkin seed flour

Mineral is a chemical element required as an essential nutrient by organisms to perform functions necessary for life (Zoroddu *et al*., 2019). The increase observed in some mineral content of germinated, boiled and soaked seed flours while the reduction observed in some as well. The increased seen in the germinated ones might be due to the reduction seen in the level of anti- nutrient after germination while the decrease seen might be as a result of increase requirement for nutrients by the growing plant, thus indicating transfer of nutrients from the seed material to the growing embryo. Reduction seen in mineral content during soaking couldbe attributed to the effect of leaching. Considering the macronutrients (K, P, Mg and Ca) and micro nutrients (Zn and Fe), the seed flours are fair source of minerals and these set of minerals have been reported by Fagbemi (2007) to lower the risk of high blood pressure and prevent growth and mental retardation in humans. However, the presence of these minerals whether low or high could effectively contribute to daily mineral content requirement needed.

4.2.3 Vitamin content of unprocessed and processed watermelon, moringa and fluted pumpkin seed flour

Vitamin is an organic molecule or essential micronutrient that organisms need in small quantity for proper functioning of its metabolism. The results from this research work showed variation in vitamins content of all the seed flours, this variation maybe due to difference in species of the seeds, leaching, and reduction in antinutrients or different processing methods.

4.2.4 Amino acids of unprocessed and processed watermelon, moringa and fluted pumpkin seed flour

Essential amino acids are amino acids that are required by the body but cannot be synthesized by the body they can only be supplied in diet, however the quantity of a

dietary protein is a measure of its essential amino acids which are require for growth and maintenance (Guoyao, 2014). Non-essential amino acids are equally important as they also help in growth development. Soaking significantly increased the essential and non-essential amino acids of the three selected seed flours especially in *Citrullus lanatus* and *Telfaira occidentalis* except for tryptophan where a decrease was seen generally across the seed flours.

The increment seen in soaked seed flours of Citrullus *lanatus* and *Telfaira occidentalis* might be as a result of the hydrolylic breakdown of nutrient components during soaking, this report is similar to the one reported by Bujang and Nurul (2014) in soaking of groundnuts, soybean and garbanzo that leucine is most abundant essential amino acid in all the seed flours while glutamic acid is the most abundant non-essential amino acid.

Thus, this study revealed that the three seed flours are rich source of amino acid, this suggested that these seed flours are nutritionally high quality foods and the variation seen in the amino acids content of the seed flours might be due to the effect of processing methods and degradative ability of the hydrolytic enzymes.

4.3 Antinutrient Content of the Selected Seed Flours of Unprocessed and Processed watermelon, moringa and fluted pumpkin seed flours

In this study, the determination of antinutrients such as tannins, oxalates, phytates, saponins, cyanogenic glycosides and trypsin inhibitors was of great interest because in foods, they tend to hinder efficient digestion, absorption and utilization of some nutrients and thereby reduce their bioavailability and nutritional quality leading to poor growth effect (Abdullahi and Abdullahi, 2005).

Germination, boiling and soaking tend to lower antinutrient content in all the seed flours when compared to their respective unprocessed seed flour, this reduction in antinutrient content in respect to processing methods was also reported by Adebayo (2014) which

could be as a result of leaching of the antinutrient in water especially phytate since is water soluble. Tannins reduction during germination might be as a result of formation of hydrophobic association of tannins with seed proteins and enzymes while decrease in phytate content during germination could be as a result of phytase activity as reported for other geminated cereals (Abdullahi and Abdullahi, 2005). These processing methods of seeds have been reported to enhance the permeability of the seed coat, soften the cotyledon and help in leaching out of antinutrients (Sharma *et al.,* 2012). Oxalate and phytate contents reduction as a result of soaking, boiling and germination of the seeds may result to bioavailability of essential minerals like calcium, magnesium and iron improvement (Grosvenor and Smolin, 2002; Akindahunsi and Salawu 2005). Grosvenor and Smolin, (2002) reported that tannins and trypsin inhibitor reduction may bring about protein digestibility, better bioavailability and utilization improvement of amino acid contents in the flour protein. On a general note, reduction of antinutrients properties in this research work by soaking, germination and soaking is essential for nutritional quality of the flours improvement and for enhancement of effective utilization of the full potential of the flour as a source of protein.

4.4 Functional Properties of Selected Seed Flours of Unprocessed and Processed watermelon, moringa and fluted pumpkin seed flours

Functional properties are the intrinsic physico-chemical properties that reveal the complexity, interaction between the composition, structure, confirmation and physico-chemical properties of protein and other food components and the nature of environment in which these are associated and measure (Suresh *et al.,* 2015).

Water absorption capacity (WAC), serve as index for the maximum amount of water a product can absorb and retain, and is as well importance in softening and increase digestibility (Ijarotimi and Keshiro, 2012) while oil absorption capacity (OAC) reveal if

a product will be suitable in facilitating enhancement in flavour and mouth feel when used in food preparation (Balogun and Olatidoye, 2010). In this study, these values were either higher or lower than the reports of other researchers, for instance, bulk density of watermelon, moringa and fluted pumpkin seeds flour were higher when compared soybean flour (0.38 g/cm^3) (Edema *et al*., 2005), but comparable to that of bambara groundnut which ranges from 0.60 g/cm^3 to 0.75 g/cm^3) (Onimawo and Egbekun, 1998). The bulk density of flour samples influences the amount and strength of packaging material; energy density, texture, and mouth feel (Udensi and Okoronkwo, 2006). For WAC, the value was higher in all the seed flours compared with soybeans (1.12 g/100 g) (Alfaro *et al.*, 2004).

Foaming capacity (FC) of all the processed seed floursin this study are lower when compare to that of the boiled *Moringa oleifera* seed flours, this result is similar to that reported by Aremu *et al.* (2006) where FC of boiled *Moringa oleifera* seed flours is higher than that of rear cowpea flours. Also highest foam stability was observed in soaked and boiled moringa flours. This suggest that soaked and boiled *Moringa oleifera Lam* seed flours may be preferred in cakes baking and whipping topping where foaming is important than other seed flours. This increment seed in boiled *Moringa oleifera* seed flours might be as a result of higher protein in dispersion thus causing lowering of the surface tension at the water air interface thereby foaming a continuous cohesion around air bubbles in the form (Kaushal *et al*., 2012).

The emulsify capacity and stability (EC and ES) are important parameters in the production of pastries, coffee whiteners and frozen desserts (Abdel-Shafy *et al.*, 2011). EC of all the seed flours ranges from16.61 % to 44.00 % while ES ranges from 16.66 % to 83.33 %, this suggest that the flours blends maybe good emulsifying agents. this may be due to both the soluble and insoluble protein (this is possible because the protein can

emulsify and stabilize the emulsion by decreasing the surface tension thereby providing electrostatic repulsion on the system) as well as other component such as polysaccharides (polysaccharides can also help in stabilizing the emulsion by increasing the viscosity of the system) (Abdel –Shafy *et al.*, 2011).

Swelling power (SP) and solubility (S) provide evidence of the magnitude of interaction between the starch chain within the amorphous and crystalline domain. SP and S of flours have been used to provide evidence for association of binding forces within granules (Oke *et al.*, 2013). When an aqueous suspension of starch granule is heated, they become hydrated and swelling take place.

Shimelis *et al.* (2006) classified starch to be highly swelling (30 % or higher in 95 ^{0}C), moderately restricted swelling (16 % to 20 % in 95 ^{0}C) or highly restricted swelling (below 16 % in 95 ^{0}C). From this study, all the seed flours are in highly restricted swelling category (below 16 % in 9 5^{0}C) and this result is similar to that of Oke *et al.* (2013) on yam flour. This low SC in this result would be desirable for the manufacture of value added products such as noodles and composite blends with cereals and it suggest that all the seed flours could be good sources of nutrient to its consumers and bring about food security in developing countries. The low value of SP and S of these seed flours might be due to protein- amylose complex formation.

4.5 Proximate Composition of Experimental Diets from Unprocessed and Processed Watermelon, Moringa and Fluted Pumpkin Seed Flours

The proximate composition of the experimental diet showed that the seed flours are rich in essential nutrients and these means these nutrients could supplement other dietary sources if the seed is served in adequate amount in diet.

Steady increase in the body weight of all the experimental rats was observed during the course of the feeding trials; these suggest that the seed flours did not exert any deteriorative effect on the weight and growth of the animals. The soaked *Citrullus lanatus* has the highest body weight increase.

4.6 Weight Changes of Rats Fed Seed Flours Supplemented Diets

A steady increase in weekly weights was observed for rats fed with control and experimental diets; although rats fed with soaked watermelon seed flour supplemented diets had higher weight gain as compared to rats fed other supplemented diets. The significant increase ($p < 0.05$) in weight gain in rats fed soaked watermelon seed flour as compared other supplemented diets may therefore suggest that watermelon may have a higher protein This result is in agreement with the findings of Angeline and Krishnakumari (2015) who reported that watermelon seeds are of high nutritional value because they are rich source of proteins, vitamins, omega 3 and omega 6 fatty acids, magnesium, zinc, copper, potassium and are high in calories.

4.7 Biochemical Parameters from Serum of Rats Fed Seed flours Supplemented Diets

Alanine aminotransferases (ALT), aspartate aminotransferases (AST) and alkaline phosphatase (ALP) are enzyme biomarkers to monitor the hepatic structural integrity, damage and aid the clinical diagnosis of liver toxicity conditions.

The enzymatic activity of ALT, AST and ALP were studied to evaluate liver malfunctions. In acute hepatoxicity. Liver enzymes levels are usually raised, but decrease overtime with prolonged intoxication due to damage to the liver (Obi *et al*., 2004)

The processed *Citrullus lanatus, Moringa oleifera and Telfaira occidentalis* seed flours revealed a significant reduction on liver enzymes when compared to the unprocessed except for ALP in boiled moringa. Imafidon and Okunrobo (2012) reported that changes in serum protein, albumin, bilirubin, ALT, AST and ALP concentrations indicate changes in the normal liver functions. The reduction seen in liver enzymes levels suggest that the seed flours did not have any impairment in the normal liver functioning. However, the significant ($p < 0.05$) increase in ALP activity observed in the serum of rats fed boiled moringa supplemented diet may not necessarily be as a result of liver damage. Raised serum ALP level usually reflects impaired excretion and bile flow like that observed during obstruction that could affect the biliary system. Increased serum levels may reflect increased synthesis or inadequate excretion as in conditions leading to increased biliary pressure. Thus, the increase observed could not have indicated hepato biliary obstruction; rather extra hepatic sources (e.g. bone) may have been implicated (Adedeji *et al.*, 2017). Generally, the decrease in liver enzymes (AST, ALT) and bilirubin suggests that these seed flours have little or no deleterious effect on the liver.

Creatinine and urea are the diagnostic tools for kidney functions because they are easily measured byproducts of muscles metabolism that are excreted unchangeable by kidney (Allen, 2012), creatinine blood levels rise, if the filtration in the kidney is deficient. The level of creatinine in all the groups significantly decrease when compared with the control. This might be due to the seeds antioxidant and phenol content that have scavenging effect on the free radicals and it may indicate that functioning of the kidney is normal, this is in line with Ndong *et al.* (2007) who stated that seeds restored the normal renal function and histology of kidney with no pathological changes. Significant changes are observed in the levels of urea, sodium, potassium and chloride across all the

seed flours and it might be as a result the seeds been subjected to different processing methods.

4.8 Haematological Parameters from serum of Rats Fed seed flour Supplemented Diets

Result obtained showed that the value of HB, PCV, MCHC, RBC, increases significantly ($p<0.05$) when compared to that of the unprocessed seed flour for *Citrullus lanatus.* This increment is an indication that the processing methods significantly improved the quality of the *Citrullus lanatus* seed flour, improvement maybe due to among other factors inactivation of the antinutrient factor present in the seed flour and transformation of some of the component nutrients to non-toxic, more readily digestible absorbable form (Wagner *et al.,* 2007).

While decrease seen in some haematological parameters in this study might be as a result of the anti-nutritional factor. Some of these antinutrients are known for their negative effect on some haematological parameters for instance, saponins are known to cause erythrocyte haemolysis and reduction of blood (Jimoh *et al.,* 2014). This is related with the findings done by Osuigwe *et al.* (2005) that nutritional toxicity is associated with anaemia and that some antinutrient found in seeds severely reduced blood PCV and Hb concentrations.

However, it is important to note that inspite of reduction in haematological parameters levels of the seed flours at different processing methods when compared with the control, they were still within the normal range reported by Musa and Omoregie (1999), this may be responsible for the non mortality observed in this work. Suggesting that the animals were not anaemic.

4.9 Lipid Profile from Serum of Rats Fed Seed Flour Supplemented Diets

Lipids are fats and fatlike substances that are important part of cells and energy source. A lipid panels measure the level of specific lipids in blood to help assess risk of cardiovascular disease. The result obtained in this study revealed that serum TG and LDL of all the processed group of *Citrullus lanatus* decreases when compared to the unprocessed seed, citrulline is present in *Citrullus lanatus* seed (Reetapa *et al*., 2015) which may be the possible reason of improving the lipid profile parameter in this group. Previous report had it that L-citrulline can improve endothelium-dependent vasorexalation and relax arterial smooth muscle by an effect on cyclic GMP.

There were no significant different in cholesterol level, TG, HDL and LDL in the experimental rats fed with *Moringa oleifera* seed flour supplementary diet, this is in line with previous research that suggest the ameliorative effect of *Moringa oleifera* seed on lipid profile. No significant different advocate that *Moringa oleifera* at different processing levels could correspondingly act as hypolipidemia, hypolipoproteinmia and hypocholesterolemia agent and this is supported by previous findings that every part of *Moringa oleifera* plant (seed, leave, stem) lowered the principal marker linked with coronary artery diseases and acute myocardial infarction in high diet rats (Jiki *et al*., 2018) significant elevation was seen in HDL concentration of rat fed soaked *Telfaira occidentalis* seed flour supplemented.

4.10 Effect of Seed Flours Diets on the Histopathology of liver and kidney of the experimental rats

Histological examinations of tissues are used as complementary evidence to biochemical evaluations in revealing any distortion or damage to the structures of tissues (Akanji *et al*., 2008)**.** From this study, histomicrographs of the liver and kidney of rats fed *Citrullus lanatus, Moringa oleifera* and *Telfaira occidentalis* seed flour

supplemented diets revealed no alteration or pathological effect on the morphology of liver and kidney of the experimental rats except for the group fed with unprocessed *Telfaira occidentalis* seed flour. This is supported with the research reported by Mokrane *et al*. (2020).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study showed that *Citrullus lanatus, Moringa oleifera* and *Telfaira occidentalis* seeds have high nutritive values and were found to be good sources of protein, fibre and carbohydrate both in their unprocessed and processed state. However, the presence of antinutrients (tannins, phytate, oxalate, saponin, trypsin inhibitors and cyanogenic glycoside) in the unprocessed state affected the bioavailability of the nutrients.

This study also suggests that these seeds could be good in baking and can as well be incorporated into other flours to improve nutrient quality of foods.

It also revealed that soaked watermelon seed flour supplemented diet bring about significant increase in weight changes of experimental rats compared to others.

It showed a better outcome in terms of heamatological parameters, decreased liver biomarkers and normal in kidney and liver histoarchitecture.

Generally, this study also showed the hepatoprotective activity of the selected processed seeds on the liver. Therefore, these underutilized seeds may be a promising alternative to conventional foods and their inclusion in diet might boost the nutrient quality of foods.

5.2 Recommendations

i. For optimum nutritional quality of the flours, watermelon seed should be boiled or soaked; moringa seeds should be geminated and fluted pumpkin seed should be soaked and germinated.

ii. It is recommended that these underutilized seeds should be in diets, inorder to exploit their nutritional potentials.

iii. Proper processing and packaging are recommended in order to improve the acceptability these selected seeds.

iv. Further research could be done on these seeds using other processing methods viz: freezing, roasting, fermentation, irradiation, pasteurization, genetic engineering, curing and so on.

5.3 Contribution to Knowledge

This study showed that *Citrullus lanatus, Moringa oleifera* and *Telfaira occidentalis* seeds have high nutritive values and were found to be good sources of protein (18.77 %, 32.61 %, 25.70 %) fibre (19.36 % for watermelon seed and 13.61 % for moringa seed) and carbohydrate (40.41 %, 35.61 %,17.88 %) both in their unprocessed and processed state. However, the presence of antinutrients (tannins, phytate, oxalate, saponin, trypsin inhibitors and cyanogenic glycoside) in the unprocessed state affect the bioavailability of the nutrients.

This study also suggests that these seeds could be good in baking and can as well be incorporated into other flours to improve nutrient quality of foods.

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APPENDICES

Appendix A

Pictures of Germinated Watermelon (*Citrullus lanatus)* Seeds

Appendix B

Picture of Germinated Moringa (*Moringa oleifera Lam.*) Seeds

Appendix C

Picture of Germinated Fluted Pumpkin (*Telfaira occidentalis*) Seeds