**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY**

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# Evaluation of the Mineral Composition, Antioxidant Properties, Phytochemical and Anti-nutrient Composition of African Palmyra Palm (*Borassus aethiopum*) Fruit Flour

**BY**

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**(APRIL, 2018)**

# EVALUATION OF THE MINERAL COMPOSITION, ANTIOXIDANT PROPERTIES, PHYTOCHEMICAL AND ANTI-NUTRIENT COMPOSITION OF AFRICAN PALMYRA PALM (*Borassus aethiopum*) FRUIT FLOUR

BY

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(BSc. Food Processing Engineering)

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**DECLARATION**

I hereby declare that I have wholly undertaken the study reported herein under the supervision of Dr. Agbenorhevi, K. Jacob and that except portions where references have been duly cited; this thesis is the outcome of my research.

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## ABSTRACT

African palmyra palm (*Borassus aethiopum*) grows widely across Africa. Previous studies indicated its fruit flour has a great potential in food applications. However, there is limited information on the mineral composition, antioxidant properties, anti-nutrient composition and phytochemical composition of the flour. The main objective of this work was to investigate the effect of drying methods on the above mentioned properties of the African palmyra palm flour. Previous works carried out on the flour however indicated that drying had an influence on some quality attributes of the flour. The fresh fruit pulp was obtained, freeze dried, oven dried, solar dried and milled into flour (particle size: 450 microns and below). Phytochemical screening, mineral analysis, anti-nutrient analysis and antioxidant analysis were conducted on the flour obtained. The flour had total phenols (1518.00 - 3896.71 mg GAE/100g), potassium (237.00 - 276.73 mg/100g), magnesium (211.61 - 293.62 mg/100g) and saponin (36.10 – 55.62 g/100g). The phytochemical screening indicated the presence of many phytochemicals including glycosides. Drying had a significant effect on the analysed composition of APP flour.

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# CHAPTER ONE

## 1.0 Introduction

## 1.1 Background

*Borassus aethiopum* is an *Arecaceae* and is also known in Africa as the mother of trees or as the Savannah's guard (Ali *et al*., 2010). *Borassus aethiopum* goes by other common names such as ron palm, borassus palm, African fan palm, palmyra palm and or deleb palm (Orwa *et al*., 2009). In Ghana, the Akans refer to it as "Mmaa kube".

This species is documented as being native to countries like Benin, Burkina Faso, Congo, Cote d'Ivoire, Ethiopia, Gambia, Ghana, Guinea, Kenya, Liberia, Nigeria, Sierra Leone, just to mention a few (Orwa *et al*., 2009). As in other countries, African Palmyra palm grows in the wild in Ghana but more visible within some parts of the Volta, Eastern, Ashanti and Brong Ahafo regions. In the Abrimasu Forest Reserve in Mampong Forest District of the Ashanti Region of Ghana, where a survey was conducted by Siaw *et al*. (2014), the population density of the Palmyra is reported to be about 18-61 trees per hectare.

The African fan palm is an attractive palm and every part of the tree can serve any of the socio-cultural, economic and cultural needs of human kind (Siaw *et al*, 2014). The leaves are useful in the basket and mat industries whereas the trunk is useful in the construction of bridges telegraphic poles due to the toughness and termite resistant nature of the wood (Sarkodie *et al*., 2015). According to Sambou *et al*. (1992), upon ripening, the mesocarp is fleshy and can be consumed by

grilling, boiling or mixing with sugar or honey. The roots are used in traditional medicine to cure various ailments including asthma.

Over the years, various drying methods, whether conventional or with advanced technology have been engaged in the preservation of many grains, fruits and vegetables to extend their shelf life. The fruits of *Borassus aethiopum* is noted to having a high moisture and nutrient content. These attributes make the fruit ideal for spoilage microorganisms to thrive leading to post harvest loss during peak seasons. In order to mitigate the effects of this post-harvest loss, drying of the fruits can be employed to remove or lower the moisture content so as to avoid or slow down food spoilage by microorganisms. According to Ogoreyo *et al*. (2011), the removal of water by heat can either increase the concentration of some nutrients by making them more available or decrease the concentration of others.

**1.2 Problem Statement**

According to Ali *et al*. (2010), *B. aethiopum* bears fruits every 8 months and produces between 50 and 150 fruits (50 to 175 kg), depending on the size of the fruits. Nonetheless, only about 30-40% of the fruit is utilized leaving almost 70% to go as waste. The high level of losses recorded is mainly as a result of the high moisture content (70 – 81%) and underutilization of the fruit. Findings from a study conducted by Abe-Inge *et al*. (2017) indicated *Borassus aethiopum* fruit flour has great potential applications in the food industry. However there is currently no study on the mineral content, phytochemical and antioxidant properties of the flour produced from the African Palmyra palm fruit in Ghana.

### 1.3 Justification

This study will provide information on the mineral content, phytochemical and antioxidant properties of *Borassus aethiopum* fruit flour. This may help increase and diversify the use of the *Borassus aethiopum* fruit flour in the food industry, which in turn, will reduce postharvest losses and/or wastage of *Borassus aethiopum* fruits.

### 1.4 Objective

This work seeks to evaluate the mineral composition, antioxidant properties, phytochemical and anti-nutrient composition of *Borassus aethiopum* fruit flour.

### 1.5 Specific Objectives

- To determine the mineral content (Ca, Fe, P, K, Mg, Na, Zn, Mn) of *Borassus aethiopum* fruit flour
- To determine the total phenol content of *Borassus aethiopum* fruit flour
- To determine the anti-nutrient composition (tannins, oxalate, saponin, alkaloids) of *Borassus aethiopum* fruit flour
- To determine the DPPH(2,2-Diphenyl-1-picrylhydrazyl) scavenging activity of *Borassus aethiopum* fruit flour
- To assess the effect of freeze, hot air oven and solar drying methods on the above mentioned factors of *Borassus aethiopum* fruit flour.

# CHAPTER TWO

## 2.0 Literature Review

### 2.1 Origin, Distribution and Characteristics of *Borassus aethiopum* Mart.

The plant, *Borassus aethiopum* as documented by Siaw *et al*. (2014), was first described in India in 1753 and only much later in Africa, however botanists believed that it originated from Africa. Literature has it that, the existence of *B. aethopium* in the West African states can be attributed to the dispersion caused by the migration of elephants (lovers of the fruit) and slave traders. Barot and Gignoux described *B. aethiopum* as a common palm in West African humid savannas, though it can also be found in drier areas, the plant is restricted to riversides.

The plant is distributed over countries like Benin, Niger, Togo, Ghana, Cote d'Ivoire, Gambia, Senegal, Burkina Faso, Mali, Mauritania, Nigeria and Tchad (Ouinsavi *et al*., 2011). Cameroon, Ethiopia, Central African Republic, Democratic Republic of Congo, Niger and Sudan are also some natural distribution areas as documented by Ali *et al*. (2010). Dense populations of Borassus aethiopum are found in the southern and southeastern parts of Senegal close to rivers and lakes where the groundwater level is high (Sambou *et al*., 1992).

In Cameroon, the distribution range is vast, with some located between the Lake Chad basin and the foot of Mount-Cameroon mountain passing through the Benoue basin. Other prominent populations in the Northern regions can be sighted around Poli and Rey-Bouba whiles in the Adamawa highlands plant density are significant in the Vina valleys and Mbere division (Ali *et al*., 2010).

*Borassus aethiopum* is a dioecious, tall, and solitary and palm with a height ranging from 20 – 30m (Barot and Gignoux). According to Barot and Gignoux, seed germination of the *Borassus aethiopum* is remote – tubular, implying that the embryonic axis is pushed into the soil to varying depths by downward extension of the cotyledonary petiole from which the seedling develops at varying distances approximately 40cm from the seed (Baskin and Baskin). The flowers are yellowish with the male and female flowers appearing on separate trees. The male flowers are abundant and small whereas the female flowers are larger. The leaves appear bluish-green in colour, and are fan shaped with petioles of 1.5 to 3 m long. The stem is massive and covered with leaf bases abscising clearly in older individuals. (Ouinsavi *et al*., 2011; Orwa *et al*., 2009)

A study conducted in Cameroon by Ali *et al*. (2010), stated that, the trees of *B. aethiopum* are found in groups of 60-120 feet per hectare and produce about 150 - 350 fruits each per season. The fruits weigh 1.5-2.0 kg thus producing an estimated 15-40 tons per hectare. Siaw *et al*. (2014), recorded a population density of 18-61 trees per hectare within the Abrimasu Forest Reserve of Ghana. The tree can withstand high temperatures and will grow in areas with rainfall less than 500 mm/yr if the groundwater table is high.

The edible fruits of *B. aethiopum* are gathered in tightened bunches, containing each two to three cores surrounded by a fibrous flesh. They are ovoids or smooth globulous and fibrous drupes, which measure from 15 to 20 cm in diameter. When matured, they look yellow, orange or slightly reddish (FAO, 1993, Ali *et al*, 2010).

According to Ali et al., 2010, the fruits pulp has an output in flesh of 38% of the fruit total weight. It is rich in total sugars and in fibers with average contents of 25.5% (dry matter). It also contains essential minerals and vitamins with the following contents (mg/100g, fresh matter: calcium (107- 108); phosphorous (560-567); magnesium (20-21); iron (2- 2.2); Vitamin C (134 – 171); total carotenoids (26 – 28).

A report by Ali *et al*. (2010) indicates that the Borassus aethiopum, although a multi-purpose plant, with diverse usefulness among both urban and rural populace of Cameroon is still highly under-exploited. Other findings in literature also make mention of *B. aethiopum* being a vital crop amongst the Senegalese rural areas due to its varied usefulness , however the plant population is being threatened due to destructive agricultural practices and lack of management ( Sambou *et al*., 1992)

## 2.2 Current Uses of *Borassus aethiopum* Mart in Africa

### 2.2.1 Food Applications of *Borassus aethiopum* Mart.

Different parts of the *B. aethiopum* plant serve as delicacies particularly in the rural areas where the populations are dominant. According to Aguzue *et al*. (2013), the young germinating shoot or the hypocotyls known as Muruchi, is an important source of food for the rural folks in Northern Nigeria. Muruchi, which appears about two months after the seeds are grown, is harvested, being very starchy, and considered of great value in times of famine. Similarly in Cameroon and Niger, the hypocotyls, usually referred to as “Batchi” and “Miritchi” respectively is a product which has a significant taste and its aspect when cooked

or roasted resembles the roots of manioc. It is then converted into flour for the preparation of the paste or the 'fufu' (Ibrahima, 2005; Ali *et al*., 2010).

A study conducted by Siaw *et al*. (2014), also revealed that 54% of his correspondents from the selected fringe communities of the forest reserve consume the hypocotyls of the B aethiopum either as food (Tuekokoo) or as palm wine. Tuekokoo' is boiled maize with juice obtained from the fruit of *Borassus aethiopum*

Juice extracted from the ripe fruit, which has been described by Ali *et al*. (2010) as sweet, viscous and scented is used in the production of millet flurry or in the preparation of wafers of millet. The fruit has sugars, provitamin A and vitamin C. In Senegal, the fleshy mesocarp upon ripening can be prepared and eaten in different ways: grilled, boiled or mixed with honey. The young gelatinous endosperm is deemed a nutritious snack. The premature folded leaves of the seedling are said to be tasty and thus are collected from below the soil and eaten as reported by Sambou *et al*. (1992).

The sap, which is also very high in sugar, is tapped from near the apical meristem at the base of the palm heart (Bismuth and Menagi 1961; Sambou *et al*., 1992). The fresh sap can be used as yeast, made into vinegar, boiled after the extraction process to produce sugar or further fermented to produce an alcoholic beverage (Orwa *et al*., 2009 and Sarkodie *et al*., 2015). The fruits have a large, fibrous pulp (around 500 g each) and can be consumed raw or cooked, preferably with rice (Orwa *et al*., 2009).

### 2.2.2 Common Non-Food Uses of *Borassus aethiopum* Mart.

Throughout literature the trunk/ stem of Borassus aethiopum is described as a hard wood that is highly resistant to decay, termite attacks, fire and the damaging effects of seawater. These attributes, coupled with its excellent working-properties makes it the wood of choice for the construction of bridges, houses, shower cabins, boards for making floor and walls, just to mention a few. In Mozambique, the trees are used to make dugout canoes whiles in Uganda they are cut and hollowed out to make beehives. Door frames, roof materials, tool handles and drums are some derivatives of the tree (Orwa *et al*., 2009; Sambou *et al*., 1992)

Other useful parts of *Borassus aethiopum* are the leaves and flowers. The leaf fibers are extracted and used for brooms and baskets whiles the leafstalk endings can be soaked in water to provide fibres that are used as sponges or filters. The leaf petioles are found useful in the domain of arts and crafts and also employed to create fences around houses and pastures. The blade or segments of the blade are useful in the making of thatch, mats, rugs and bathtub (Sambou *et al*., 1992). The male flowers, in addition to being used as soil fertilizers are also considered an excellent source of fodder due to their appreciable level of nutrient content being similar to those of groundnut and cowpea haulm (Ouinsavi *et al*., 2011).

### 2.2.3 Medicinal Benefits and Applications of *Borassus aethiopum*

Studies conducted reveals that *Borassus aethiopum*, apart from its usefulness in food applications is also used widely for medicinal purposes. According to Sambou *et al.* (1992), an infusion of the roots is used to treat miscellaneous ailments such as stomach ache, throat infections, bronchitis and syphilis. The

flower-bearing branches are employed in the treatment of venereal diseases. A mixture of the stamens with Shea-butter is used to heal wounds whiles the mesocarp of the ripe fruit is applied as a stimulant and against tetanus. It is said to be a very efficient remedy against intestinal parasites too. The sap tapped from the stem is believed to speed up the growth of teeth in children.

In traditional medicine, palm wine is a constituent of several aphrodisiac preparations. The flowers provide relief against aphonia, and young leaves are used to stop haemorrhage (Orwa *et al*., 2009). Like its usefulness in food applications the roots, leaves, flowers and fruits are used for multiple medicinal purposes such as treatment for sexually transmitted diseases (e.g., beign herpes), cutaneous fungal infections, and viral infections particularly measles (Sarkodie *et al*., 2015) The flowers are useful in the treatment of impetigo, whereas the roots, for asthma treatment. Again, Sarkodie *et al*. (2015) report that some inhabitants of several communities in Ghana apply the edible flesh seed in the treatment of diarrhea and muscle tremor in indigenous poultry species including Guinea fowls *(Numida meleagris).* Furthermore, studies conducted by Siaw *et al*. (2014) within the Mampong Forest District indicated that some respondents mix the roots' powder with shea butter to treat sore throat and bronchitis. In the rural parts of Northern Nigeria, the germinating shoot or hypocotyl usually referred to as *Muruchi* when consumed is perceived to enhance libido in women and provide aphrodisiac properties in men (Gbesso *et al*., 2016).

## 2.3 Proximate and Mineral Composition of African Palmyra Palm Fruits

### 2.3.1 Proximate Composition of African Palmyra Palm Fruits

Proximate composition includes the moisture content, fat content, ash content, protein content and fiber content with nitrogen-free extract being estimated by subtracting the sum of these five percentages from 100. Proximate composition together with the mineral composition of a particular product is termed the nutritional composition of that particular product. Proximate composition may vary depending of the kind of food, the species, the origin, the climate conditions in which the food was grown and many others. In a review conducted by Jansz *et al*. (2002), palmyrah fruit pulp (*Borassus flabellifer* L.) consisted of 75-80% moisture. Below is the proximate composition of palmyrah fruit pulp in two different studies.

**Table 2.1: Proximate composition of Palmyrah Fruit Pulp (Balasubramanium *et al.*, 1999); Jeyaratnam (1986)**

| Constituent (per 100g) | Study 1 | Study 2 |
|---|---|---|
| Moisture (g) | 77.2 | 79.1 |
| Energy (kcal) | 87 | - |
| Protein (g) | 0.7 | 2.8 |
| Fat (g) | 0.2 | 1.0 |
| Total carbohydrate (g) | 20.7 | 18.5 |
| Sugars | - | 14-16 |
| Crude fiber | - | 1.5 |
| Ash | - | 4.3 |

Umar *et al*. (2002), reported that the proximate composition of *Borassus aethiopum* (African palmyrah palm) shoots showed 56.33% w/w moisture,

11.2%DW crude fiber, 6.9%DW crude protein and 8.1%DW available carbohydrate.

In the work of Oryema and Oryem-Origa (2016), the proximate composition of *Borassus aethopium* pulps were determined on wet matter (WM) basis and dry matter (DM) basis and the results is shown in Table 2.2 below.

Djibrilla (2006) also recorded the proximate composition of flour from *B. aethiopum* fruit pulp with matter content of 91.79%; total ash content of 2.7%; total lipid content of 0.16%; crude protein of 4.23%; crude fibre of 29.75%.

**Table 2.2: Proximate composition of *B. aethiopium* pulps on DM and WM basis**

| Composition (per 100g) | Dry matter (DM) basis | Wet Matter (WM) basis |
|---|---|---|
| Dry matter | 19.70 g | 80.30 g |
| Crude fiber | 4.50 g | 0.76 g |
| Crude fat | 2.60 g | 0.51 g |
| Ash | 3.3 g | 0.64 g |
| Proteins | 4.24 g | 0.83 g |
| Carbohydrates | 43.5 g | 8.54 g |
| Energy | 367.97 kcal | 72.15 kcal |

Source: Oryema and Oryem-Origa (2016)

### 2.3.2 Mineral Composition of African Palmyra Palm Fruits

Minerals are the inorganic components required by the body in small amounts for a variety of functions. They are commonly referred to as micronutrients, that is, they are needed in minute quantities by the body. Micronutrients are known to play an important role in the metabolism and physiological activities in the human body. Each type of mineral plays a specific role in the body and therefore are required in different quantities depending on the age, sex, the physiological state of the individual, the health conditions and others. Minerals such as potassium, sodium, calcium, magnesium, chloride, phosphorus are needed in larger amounts in the body. Others are needed in smaller quantities and are referred to as trace minerals, e.g. iron, zinc, iodine, fluoride, selenium, copper, manganese, chromium, etc.

Minerals are important co-factors found in the structure of certain enzymes and are indispensable in numerous biochemical ways. They are also needed by the body for the construction and maintenance of bone and normal function of nerves and muscles. Some minerals such as phosphorus is an important constituent of adenosine triphosphate (ATP) and nucleic acid and is also for acid-base balance, bone and tooth formation. Iron plays a crucial role in the oxygen-carrying pigment of red blood cells (haemoglobin) and it is also an important component of cytochromes that function in cellular respiration. Sodium, potassium and chlorine are important in the maintenance of osmotic balance between cells and interstitial fluids. However excessive intake of these minerals may upset homeostatic balance and cause toxic side effects. For instance high intake of

sodium increase the risk of hypertension and also high iron intake can cause liver damage (Soetan *et al*., 2010).

**Table 2.3: Compositions of macro and micro mineral of the pulps on Dry Matter and Wet Matter basis**

| Composition (per 100g) | Dry matter (DM) basis (mg/100g) | Wet Matter (WM) basis (mg/100g) |
|---|---|---|
| Na | 17.8 | 12.0 |
| K | 142.4 | 42.6 |
| Mg | 64.77 | 19.4 |
| Fe | 3.15 | 0.62 |
| Zn | 0.82 | 0.66 |
| Cu | 1.3 | 0.26 |
| Ca | 41.81 | 33.6 |

Oryema and Oryem-Origa (2016), the mineral composition of *Borassus aethopium* pulps were determined on wet matter (FM) basis and dry matter (DM) basis and the results is shown in Table 2.3 above.

### 2.3.3 Phytochemical Composition of African Palmyra Palm

It has been proved that fruits from *Borassus aethiopum* have a number of phytochemicals that serves as nutraceuticals while others such as alkaloids, phytates and tannins are considered as antinutrients above their tolerable levels. Phytochemicals are non-nutritive chemicals produced by plants and have the potential to prevent diseases such as colon rectal cancer and some other cancer (Jaiswal, 2012). These phytochemicals, some have biological significance as in carotenoids or flavonoids but are not established as essential nutrients. There are about 4000 different known phytochemicals. Phytochemicals that are found in foods include flavonoids, phenols, terpenoids, tetrapenoids (carotenes), xanthophyll, triterpenoid among others. According to Ahmed *et al.* (2010),

*Borassus aethiopum* hypocotyle axes flour has appreciable amount of carotenoids than it is contained in palm frees fruits. Also, the anti-nutritional factors such as phenolic compounds, tannins, phytates, oxalates and saponins are present in the flour of *Borassus aethiopum* Mart but at different levels. All these antinutrients affects the bioavailability of some essential nutrients such as zinc, proteins and calcium in the body.

### 2.3.4 Antioxidant Properties

Antioxidants are molecules that protect the cells or system against damage caused by oxidants via oxidation reactions. Antioxidants exist both in natural or synthetic (artificial).Natural antioxidants include phenolic compounds such as catechin, rutin, procyanidins and epicatechin which have been identified in the okra plant and many other plants (Khomsug *et al*., 2010; Geng *et al*., 2015). Carotenoids as well as vitamins A and C are also antioxidants commonly found in plant materials. Antioxidant act as free radical scavengers in biological cells.

The ability of antioxidants to terminate free radical chain reactions and prevent cell damage makes them useful in preventing diseases such as cancer, heart attacks, unhealthy aging, macular degeneration which result from cell damage caused by high free radical levels in the body (Amic *et al*., 2003). The activity of antioxidants may be dependent on their concentration as well as the medium of reaction. Different concentrations of antioxidants are present in the various food materials, both processed and raw, in existence including the fruits of African Palmyra palm.

According to Ali *et al*. (2010a) who studied the physicochemical properties of African Palmyra palm fruit, the fresh pulp of the fruit contains about 274.56 mg/100g total phenols, 26.61 - 27.42 mg/100g carotenoids and 134.82 – 171.33 mg/100g vitamin C. However, in another study conducted by Ali *et al*. (2010c), the dried African palmyra palm fruit pulp contained 79.14 – 81.07 mg/100g of carotenoids. Also, a 70 % ethanolic extract prepared from the African palmyra palm fruit is reported to have concentration dependent total phenol content and antioxidant activity (Sarkodie *et al*., 2014). However, they reported the extract contains up to 73.65 mg TAE/g total phenols and 329.4 mg vitamin C equivalent per gram of antioxidant activity. These indicate African palmyra palm fruit pulp is a rich source of antioxidants such as phenolic compounds, vitamin C and carotenoids.

#### *2.3.4.1 Antioxidant Activity*

The occurrence and essence of oxidation in food and food products as well as in the body has being a major concern to scientists. Even though oxidative activities are necessary in the body, but the free radicals generated are of bad effect to the body and need to be scavenged (Antolovich *et al.,* 2001). Due to this scientists have researched on the various sources of antioxidants and effective methods in determining the antioxidant activities of these sources. Several methods that are used and proven to be efficient in determining the antioxidants activity from both plant and animal sources include, Peroxide Value test, Anisidine Value, $ABTS^{+}$ assay and DPPH free radicals assay among others.

The antioxidant assay, DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method depends on electron-transfer which generates a violet coloured solution in ethanol (Huang *et al.,* 2005). DPPH free radical are stable in room temperature and their oxidation activities are reduced when in contact or presence of an antioxidant compound thereby rendering the ethanol solution colourless. This assay gives a fast, reliable and easy evaluation of an antioxidant's activity using spectrophotometry (Huang *et al.,* 2005). The reduction effect by the DPPH free radicals is evaluated based on their reduction in absorbance at 517nm in the presence of antioxidants (Syed *et al.,* 2013).

This reduction in absorbance of DPPH free radical, caused by antioxidants present, advances due to the interaction between these antioxidant and radicals and leads to the scavenging of radicals through hydrogen donation(Syed *et al.,* 2013). This reaction is characterised by the change in colour from violet to yellow. Thus, the DPPH mostly serves as a substrate in determining an antioxidant's antioxidant activities (Edamatsu *et al.,* 1989).

#### *2.3.4.2 Phenols as Antioxidants*

Phenols as antioxidants act as terminators of free radicals and may function as metal chelators due to their characteristics of their chemical structure. Some phenolic compounds and their derivatives are very effective as antioxidants. Phenols such as Flavonoids are one of the antioxidants that are considered to be potent but its usage in food is still under investigation (Fereidoon *et al.,* 1992). The use of phenols as antioxidant in food is regulated by some status. Phenolic antioxidants are very good hydrogen /electron donors and also their radical

intermediates are relatively stable as a result of resonance delocalization and they have no place for oxygen molecules to attach (Belitz and Grosch, 1986). Phenols which are considered as excellent antioxidants include isoflavones, phenolic acids, robinetin, myricetin, well as chalcones that easily cyclise in acidic environment and has been proven to be effective antioxidants and they are natural precursor of flavanones and flavones (Fereidoon *et al.,* 1992). Originally phenols are inactive as an antioxidant but the replacement of the *para-positions* with alkyl groups and hydrogen atom with the *ortho-* upsurges the electron density of the OH group through an inductive effect, therefore improving their antioxidant activities toward lipid radicals (Gordon, 1990). The replacement at the *para-position* with an ethyl or -butyl group of the phenol rather than a methyl group improves its antioxidant activities but these activities can be reduced when the alkyl groups are branched or in chains (Gordon, 1990).

**2.4 Beta-Carotene**

Carotenoids are relevant pigments found in fruits and vegetables. They are responsible for the red, orange and yellow colours in plants some fishes and birds (Pfander, 1992). Over 600 carotenoids occur in nature, some include, capsanthin, alpha-carotene, beta-carotene, lutein and lycopene (Bendich**,** 1993). Beta-carotene is a major carotenoid that is required in most diets as it is important to the body. It is yellow-orange in colour and found abundantly in fruits and vegetables such as mango, pawpaw, orange, carrot, pumpkin, lettuce and cabbage (Lachance and Fisher, 1990). Beta-carotene serves as a vitamin A precursor, as it is converted into vitamin A in the body. Deficiency of vitamin A in diets can lead to children

becoming susceptible to diarrhoea and measles or other conditions such as xerophthalmia and keratomalacia (WHO, 1982; Guthrie and Picciano, 1995). Beta-carotene as an antioxidant scavenge free radicals in the body as a result, aids the prevention of cancer growth and protect the body against macular degeneration and cataract (Krinsky and Johnson, 2005; Agte and Tarwadi, 2010). According to Omayma and Abdel, (2013), a relationship has been established between the intake of beta-carotene and an increase in CD4 count in HIV patients thus, it acts as an antibody formation stimulator. Since the human body cannot synthesise carotenoids (Paul and Peter, 2004), it is advisable to consume foods rich in carotenoids to be able to obtain adequate amounts of carotenoids (especially beta-carotene) in order to escape its deficiency related diseases.

**2.5 Antinutrients**

Antinutrients are secondary plant metabolites that are produced mainly for defense against predation as well as harsh environmental conditions. They however interfere with the bioavailability of nutrients in the body when consumed at concentrations beyond acceptable limits. Antinutrients reduce the bioavailability of nutrients including proteins, minerals and vitamins by interference with their digestion, absorption and metabolism (Gemede and Ratta, 2014). Antinutrients may act as enzyme inhibitors or chelating agents for nutrients. As inhibitors, the antinutrients alter the enzyme/substrate molecular orientation required for catalysis to proceed hence interfering the normal digestion and metabolism process of the related nutrient. Antinutrients when consumed at acceptable levels could be beneficial to the body (Ugwu and Oranye, 2006) where

they act as antioxidants to reduce the risk of cancer or as anti-inflammatory agents. Some anti-nutrients include tannins, oxalate, phytate, alkaloids, saponins, cyanogenic glycosides and goitrogens according to Akande *et al*. (2010).

### 2.5.1 Oxalate

Oxalate as plant secondary metabolite is a salt formed from oxalic acids and binds to calcium, magnesium, sodium and potassium by chelation and reduces its bioavailability (Liebman and Al-Wash, 2011). Oxalates are secluded in plants cellular compartments but come into contact with nutrients in the digestive system when the food material is processed and/or digested as reported by Noonan and Savage (1999). Unlike most oxalates which are soluble, calcium oxalate is insoluble and at high concentrations form sharp-edged crystals which lead to kidney stone formation in the urinary tract (Nachbar *et al*., 2000). Besides, calcium binds with oxalate and leads to hypocalcaemia. This, when occurs with chelation of other minerals, renders calcium levels as well as related minerals in the body insufficient for its physiological and biochemical functions such as maintenance of strong bones and teeth as well as nerve impulse transmission (Ladeji, 2004; Noonan and Savage, 1999). This however does not occur in ruminants. The microflora in ruminants have the ability to metabolize calcium oxalates to less harmful by-products (Oladimeji *et al*., 2000; Akande *et al*., 2010). Ali *et al*. (2010) reported 0.98 mg/100g of total oxalates in the hypocotyls of *Borassus aethiopum* which is consumed by some rural Africans including Cameroonians.

### 2.5.2 Alkaloid

Alkaloids are small organic molecules made up of many carbon rings and has a carbon atom or more being replaced by a nitrogen. Alkaloids are found in plant materials and have bitter taste (Fereidoon, 2014). Some sources of alkaloids include, potatoes, coffee, cocoa beans and plants from most *solanum spp* (Ashihara and Suzuki, 2004; Ranjitha, and Sudha, 2015).

They are considered as anti-nutrients due to their tendency of impairing the normal functioning of the nervous system by their interference in electrochemical transmission, their ability to cause paralysis, rapid heartbeat, stomach, intestine, neurological problems and fatalities (Aletor, 1993; Fernando *et al.,* 2012, Gemede and Ratta, 2014). The intake of alkaloids above 20 mg/100 g have been recorded to be dangerous to humans (Saito *et al.,* 1990; Aletor, 1991).

It has been reported by Mathew *et al*., (2014), ripped areca nut contains 0.12–0.24 % alkaloid (arecoline) however, a work done by Awang, (1988), showed that, alkaloid content in areca nut could be reduced from sun drying, roasting, soaking in water and by boiling in water where soaking and boiling gave significant reductions.

### 2.5.3 Saponin

Saponins are naturally occurring glycosides found in plants. They have a bitter taste and can form lather in water (Bora, 2014). They can be found in pulses and oil seeds including groundnuts, kidney beans, soya beans, and sunflower (Jenkins and Atwal, 1994). Saponins have been associated with the destruction of erythrocytes, blood volume reduction, low nutrient absorption, poor enzyme

activities, stunted growths, nauseating feelings, vomiting and low cholesterol (Cheeke, 1971; Price e*t al.,* 1997). Their tendency to lower cholesterol comes about when they bind to cholesterol hence, prevent their absorption into the body (Sidhu and Oakenfull, 1986). Saponins are used in the beverage and cosmetic and pharmaceutical industries, however their use may be limited by their bitter taste (Liener, 2003; Shanthakumari *et al.,* 2008). Saponins have been successfully reduced by soaking raw food materials before cooking (Bora, 2014). Saponin of 2.18 ± 0.28 mg/100 g (DM) has been detected in the hypocotyle axes of *Borassus aethiopum* which is considered too low to achieve a reduction in in the absorption of cholesterol in the body per a research conducted by Malinow *et al.,* (1977) on the effect of alfalfa saponins on intestinal cholesterol absorption in rats where it was concluded 5-20 mg saponins is required to see any significant reduction in cholesterol absorption.

### 2.5.4 Tannin

Tannins are polyphenolic compounds, which binds to metal ions and macromolecules (Dei *et al.,* 2007). Tannins have antioxidant properties which have positive health effects on the body (Sridhar and Seena, 2006). However, when highly consumed, they can interrupt the activities of digestive enzymes (trypsin and amylase) and bind to proteins that leads to difficulty in digestion (van-Egmond *et al*., 1990; Aletor, 1993; Wheeler and Ferrel, 1971). They also negatively affect the taste of foods and cause stunted growth (Roeder, 1995). Tannins confer other nutritional effects by interfering with the absorption of Vitamin B12, iron and is considered as a possible carcinogen (Liener, 1980;

Butler, 1989). Boiling, de-hulling, cooking and germination has been reported to effectively reduce tannin contents (Salunkhe *et al*., 1990; Fagbemi *et al.*, 2005). Reedy and Pierson, (1994), attributed reduction in tannins through these actions to the action polyphenol oxidase or microbes the compounds. The presence of tannins of 239.76 ± 30.09 mg/100 g has been detected in the dried flour of the hypocotyl axes of *Borassus aethiopum Mart* which does not exceed the lethal dose of 300mg/100g *(*Ali *et al.*, 2010).

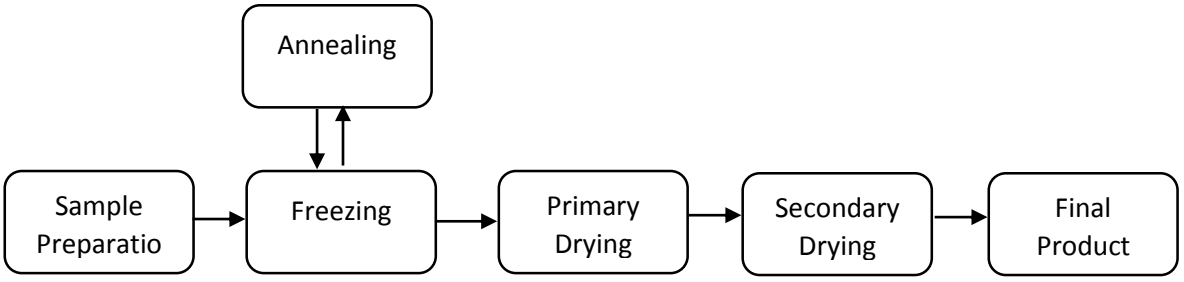
### 2.6 Drying

Drying is a technique that involves moisture removal from materials. It is often employed in the postharvest processing of food materials to increase shelf stability and maintain eating quality during storage. Drying involves heating which effects evaporation of moisture from the surface and transfer of moisture from the interior to the surface (Tiwari, 2016). The source of heat, mode of heat transfer, design and mode of operation has resulted in different drying methods in the food processing industry. The choice of drying method is influenced by cost, availability and suitability for the food material. Drum drying and spray drying methods are usually used for liquid foods (Fellows, 2000) whilst solid foods are dried by oven, sun, air, solar, cabinet and freeze drying methods.

#### 2.7.1 Freeze Drying

Freeze drying or lyophilisation involves the freezing of a product after which moisture is eliminated via sublimation (Akers *et al*., 1987). Frozen products are kept in a vacuum and heat is applied through radiation or conduction to convert

the ice into gaseous state without liquefying (Lieberman *et al*., 1989). Figure 1. shows the freeze drying cycle of a product.




(Nireesha *et al*., 2013).

**Figure 2.1 Freeze drying cycle of a product**

The freeze drying technique is used to dry products sensitive to heat and water (Nireesha *et al*., 2013). Freeze drying of products enhances their stability and portability (Carpenter and Chang, 1996; Pikal, 1998). The draw back in using the freeze drying technique is its time and energy consumption, which only could be managed through optimization of the freeze drying cycle (Franks, 1990; Pikal, 1990; Nail and Gatlin, 1993; Beals, 1997). Due to the activities involved in the regulation of temperatures, pressures and drying rates, freeze drying processes inquires a lot of expenses, hence is termed to be an expensive procedure for a better quality product compared to other drying techniques (Luther *et al*., 2004).

### 2.6.2 Oven Drying

This drying technique requires the use of a high drying temperature to enhance the drying rate and in achieving this greater amount of energy is required (Qing-guo *et al.,* 2006; Chin *et al*., 2009). Hot air drying is commonly used in drying

fruits and vegetables. The aim of hot air drying is to reduce moisture content in food thus prolonging its shelf life (Di Scala *et al.*, 2011). It operates by use of hot air coming into contact with a food material via conduction or convection, under a controlled mechanised system (in terms of the air velocity, humidity, recirculation and moisture content of the product (Arora *et al*., 2006; Marques *et al.,* 2006; Doymaz, 2008). Hot-air drying technique is related to producing quality products with a uniform, clean, and aesthetically appealing outlook, when performed under controlled and monitored conditions (Dirim and Caliskan, 2012). High temperatures or prolonged drying periods may also be responsible for undesirable flavour or colour formation, nutrient damage, poor rehydration capacity and many cases the poor bulky nature of food materials (Qing-guo *et al.,* 2006). According to Roongruangsri and Bronlund, (2016), hot air-drying at 60°C is capable of retaining colour and total carotenoid content in pumpkin powder. Drying of lemon slices via hot air drying however produces a low quality product with poor active ingredients (heat labile biological compounds (Chen *et al*., 2005; Chin *et al.,* 2009).

### 2.6.3 Solar Drying

Solar drying is a method for drying and preserving food materials that involves the raising of air temperature around a material via solar energy, above the ambient air temperature, that leads to higher temperature in a solar dryer (Fuller, 2000). It is an improvement upon sun drying method where elimination of contamination, intensive labour and longer drying periods is achieved (Rajkumar**,** 2007). It is being used to dry a range of products from seeds, fruits, meat, fish to

wood pieces as it is a beneficial means in tapping renewable energy from the sun (Hii *et al.,* 2012). Advantages associated with solar drying include, protection of food materials from insects, rodents, rain, dust and excessive direct exposure to the sun (Tiwari, 2016). Jain and Tiwari (2003), refers solar drying to be an attractive drying technique in reducing loss and poor quality of products dried. Solar drying techniques also seeks to provide a cheaper, reliable and available way of drying produce in most rural areas and commercial settings that produce products on a large scale (Mekhilefa *et al*., 2011; Xingxing *et al.*, 2012). Solar drying cannot be done at night or in times of a bad weather also additional bio-fuel heater may be used to provide heat at night but it only done at a cost (Tiwari, 2016).

### 2.7 Effect of Drying on Nutritional Composition

The body requires nutrients to be able to perform its daily functions especially since it cannot synthesize them on its own hence, one's diet must be able to provide sufficient nutrients to obtain energy and grow. Processing have been recorded to affect the chemical, biological and physical nature of food materials (Karel *et al*., 1993). Though drying prolong the life span of most food materials it is usually at the expense of their quality. Drying processes involving the use of heat tends to promote enzymatic or non-enzymatic reactions which is a major influence on the reduction of nutrient contents in dried food materials (Wiriya *et al*., 2009). Drying may produce favourable results such as better sensory properties and flavour developments in most food materials, however, it may also bring about vitamin loss, fat oxidation and protein denaturation (Bonazzi and

Dumoulin, 2011). Drying significantly reduces vitamin C content even if the food material is extremely high in vitamin C (Bonazzi and Dumoulin, 2011). A longer drying period tantamount to a reduction of vitamin C contents even at low temperatures, however freeze drying techniques have been found to retain high vitamin C contents in foods (Santos and Silva, 2008; Bonazzi and Dumoulin, 2011). Marty-Audouin *et al.,* (1992), reported that destruction of food pigments such as carotenoids, chlorophylls, anthocyanins, betalains through enzyme and non-enzymatic reaction is induced by drying. Beta-carotene is degraded by most drying techniques including, convection, sun and freeze-drying according to Soria *et al*., (2009). Prolong drying of food materials at high temperatures negatively affects the functionality of proteins in diets, however low heat drying may improve its digestibility (Bonazzi and Dumoulin, 2011). Drying (by use of heat) is one of the factors that initiates lipid oxidation which is responsible for the loss of fat-soluble vitamins and pigments (Bonazzi and Dumoulin, 2011). Adopting drying as preservation technique may improve or reduce the nutritional quality in most food materials, however, each trend may be influenced by the conditions food materials are subjected to, nutritional make up or the state in which the food material is in.

### 2.8 Effect of Drying on Antioxidant Properties

High moisture accounts for the perishability of most fresh products. Dehydration methods such as oven drying, solar drying and freeze drying preserves therefore mostly employed to preserve such food products. However, under unfavorable conditions, undesirable changes such as loss of heat labile biomolecules including

antioxidants (Mediani *et al*., 2014) occur. It has been reported that the most commonly affected antioxidants are phenolic compounds due to their high susceptibility to unfavorable drying temperatures (Lim and Murtijaya, 2007; Harboune *et al*., 2009). Some non-phenolic antioxidants such as lycopene are however reported to be heat stable (Kong *et al*., 2010).

It has been reported by Mediani *et al*. (2014), who studied the effect of different drying methods on some antioxidant properties of *Cosmos caudatus* that oven drying at 45°C for 4 hours caused a relatively higher decrease in total phenolic content of the plant material compared to freeze drying and air drying methods.

Freeze drying is a technique that involves drying materials at freezing temperatures. It is expected to be very suitable in retaining heat labile compounds including antioxidants in dried products. However, a research conducted on some plant materials indicated oven dried, air dried and sun dried plant materials retained higher amounts of phenolic compounds than freeze dried plant materials (Mediani *et al*., 2014; Dossou *et al*., 2014). This could be as a result of the compositional and structural differences between the various plant materials in existence.

### 2.9 Effect of Drying on Phytochemical Properties

Phytochemicals are basic plant chemicals that can offer health/medicinal benefits to man but are however are not considered as essential nutrients (Singh *et al*., 2011). They are common in fruits and vegetables and include flavonoids, tannins, saponins, cardiac glycosides, phenolics, steroids, terpenoids and alkaloids

(Suffredini *et al*., 2004; Abioye *et al*., 2013; Saxena *et al*., 2013). Phytochemicals are considered as bio-active compounds possessing antimicrobial activities (Tsuchiya *et al*., 1996; Abioye *et al*., 2013), antioxidant, anti-inflammatory and anti-allergic properties (Maikai *et al*., 2009). High temperatures used in drying destroy most heat sensitive compounds in food materials. Most phenolic compounds are degraded by high drying temperatures (Harboune *et al*., 2009). According to Abascal *et al.,* (2005), freeze drying is the best drying technique when it comes to efficient preservation of phytochemicals in plant materials, however, in many cases it failed to preserve volatiles responsible for certain aromatic properties in some plants. Freeze drying reduced the contents of some monoterpenes found in bay leaf and parsley (Diaz-Maroto *et al*., 2002). Freeze drying and air drying have also been reported to preserve tannins better as oven-drying caused its inactivation in *Eulalia villosa* (Needs) (Du Toit and Wolfson, 1996). The use of slow freezing technique employed in lyophilisation has been reported to preserve more phenols as compared to flash-freezing (Abascal *et al.,* 2005). About 70 % of polyphenols (gallic acid, catechin, epicatechin and quercetin) were retained in freeze dried red wine according to van Golde *et al*., (2004). Oven-dried grape pomace peels at 60 °C resulted in almost the same polyphenolic, condensed tannins, and antioxidant activity compared to freeze-drying, however, oven drying at higher temperatures resulted in a significant loss as reported by Laurrauri *et al*., (1997). According to Ferreira *et al.,* (2004) oven-drying affects the phenolic compounds contents either negatively or positively, however this is dependent on the species and maturation state of the plant.

# CHAPTER THREE

## 3.0 Materials and Methods

### 3.1 Source of Sample and Flour Preparation

Fresh African palmyra fruits were obtained from Congo 3 in the Ejura-Sekyedumase district. The fruits were decalyxed, washed, peeled and pulped manually using a knife. The obtained pulp was further cut into smaller sizes and divided into three portions with each portion weighing about 600 g. The three portions were dried differently using solar drying at 37–39 °C for 7 h, hot air oven drying at 60 °C for 4 h and freeze drying for 72 hrs to achieve a moisture content of less than 10%. The dried samples were milled using the kitchen blender at speed 2 whilst pausing after every 30 s for a total time of 3 minutes. The milled samples were packaged in zip-loc bags and stored in a freezer for analysis.

### 3.2 Mineral Determination

Samples were digested according to AOAC (1990) with modifications and mineral (Na, Ca, Fe, Mg, K, Zn, Cu, Mn, Pb, Cd) content determined using Atomic Absorption Spectroscopy (AAS). About 0.25 g of each flour sample was weighed into Kjedahl digestion tubes and 7.5mL of concentrated $H_2SO_4$ was added, followed by the addition of 2.5 mL of concentrated $HNO_3$. The samples were digested at 300°C for 4 hours until the solution cleared. The clear solution was cooled to room temperature and diluted with 50 mL distilled water. The diluted solution was warmed to vaporize and atomize the mineral components prior to their quantification with the Atomic Absorption Spectrophotometer (AAS).

### 3.3 Determination of Antioxidant Properties and Phytochemical Screening

#### 3.3.1 *Borassus aethiopum* Fruit Flour Extract Preparation

Methanolic extracts of the flours were prepared by weighing 20g of each flour sample into a conical flask and 50 mL methanol was added. The mixture was allowed to stand at room temperature for 48 hours with periodic manual shaking. The liquid extract was first filtered using Whatmann filter paper No.42, followed by filtration using cotton. The residue obtained was dried to obtain a powdered extract using a rotary evaporator with a water bath set at 40 °C. The powdered extract was used for both the phytochemical screening, determination of antioxidant activity and total phenol content.

#### 3.3.2 Determination of DPPH Scavenging Activity

DPPH scavenging activity was determined according to the modified DPPH assay method by Larbie *et al*. (2017). Stock solution of the extract was prepared by dissolving 10 mg of the dried sample in 1 ml of methanol. Also, stock solutions of 10 mM of standard (Ascorbic acid) and 0.5 mM of DPPH were prepared by dissolving 0.176 mg of Ascorbic acid and 3 mg of DPPH in 1mL of water and 15 mL absolute methanol respectively. The solutions were then vortexed until complete dissolution was achieved. The DPPH solution was immediately kept in the dark as it photo-bleaches in light.

In a 1.5 mL eppendorf tube, the extract was serially diluted in water to obtain a concentration range of 0.156–10 mg/mL. Hundred microliters of each concentration of the test sample was transferred into a 96 well plate. This was followed by the addition of 100 $\mu$L of 0.5 mM 2, 2-diphenyl-1-picrylhydrazyl

radical (DPPH). For positive control or standard, ascorbic acid was used at a concentration range of 0.156–10 mg/mL in distilled water. Distilled water was used as blanks. Triplicate experiments were performed. The plates were covered with aluminum foil, shaken gently and kept in the dark for 20 minutes after which the absorbance was read on a Synergy H1 plate reader at the absorbance wavelength of 517 nm. Percentage scavenging activity was determined by;

$$\% \text{ Scavenging} = \frac{[\text{Absorbance of blank (OD}\mathbf{0}\text{) - Absorbance of test (OD}\mathbf{1}\text{)}]}{\text{Absorbance of blank (OD}\mathbf{0}\text{)}} \times 100$$

The inhibitory concentration at 50 % (IC50) values, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, were determined by nonlinear regression analysis.

### 3.3.3 Determination Total Phenol Content

The total phenol content of *B. aethiopum* fruit flour extracts was determined according to the modified Folin Ciocalteau method by Larbie *et al*. (2017). Stock solution of the extract was prepared by dissolving 10 mg of each of the dried samples in 1 mL methanol. A stock solution of 5 mg/mL of standard (gallic acid) was prepared by dissolving 50 mg of it in 1 mL absolute ethanol. This was then diluted in 9mL distilled water to obtain the 5 mg/mL stock solution.

A two fold serial dilution was carried out on the gallic acid standard to obtain six different concentrations 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mg/mL. A water blank, that is, water without gallic acid, was also prepared. A two fold serial

dilution was also carried out on the extract to obtain three different concentrations (10, 5, 2.5 mg/mL). Water without extracts, was also prepared as blank.

A volume of 10 µL of the sample and gallic acid dilutions were aliquoted into a 2.0 mL eppendorf tube. Aliquots of 790 µL of distilled water were then added and this was followed by the addition of 50 µL of Folin-Ciocalteau reagent. The mixture was mixed thoroughly by vortexing for five seconds. This was followed by incubation of the tubes in darkness at room temperature for eight minutes. Afterwards, a volume of 150 µL of 7 % sodium carbonate solution was added to each tube, mixed thoroughly by vortexing for five seconds and further incubation of the tubes in darkness at room temperature was done for two hours. After the two hour incubation, a volume of 200 µL of the extract and gallic acid standard dilutions was aliquoted into wells on a 96-well plate in triplicate and absorbance read at 750 nm using microplate spectrophotometer (Synergy H1). A graph of absorbance against concentration was plotted for the gallic acid standard. The concentration of phenolics in the extract was determined using the gallic acid standard plot.

### 3.3.4 Preliminary Phytochemical Screening

Phytochemical screening on flour extracts were carried out according to the methods described in the works of Trease and Evans (2002), Onike (2010) and Tiwari *et al*. (2011).

#### *3.3.4.1 Test for Tannins*

Two hundred milligrams of each flour extract was weighed and warmed with 20 mL distilled water in a water bath for 5 minutes and filtered. To 1 mL of the extract solution, 10 mL distilled water was added and 3 drops of 1% lead acetate solution was added. The presence of colored precipitates the presence of tannins.

#### *3.3.4.2 Test for Alkaloids*

Two hundred milligrams of each flour extract was weighed was extracted with 50 mL ammoniacal alcohol (1 part concentrated ammonia: 9 parts of 95% ethanol) and filtered. The filtrate was evaporated on a water bath to dryness. The scum obtained was extracted with 1% $H_2SO_4$ and filtered. Dilute ammonia solution was added to the filtrate obtained to make it alkaline, then mixed with chloroform and shaken in a separating funnel. The chloroformic layer was separated and evaporated to dryness in a water bath. Then dried residue was dissolved in 1% $H_2SO_4$ and 3 drops of Dragendorff's reagent (potassium bismuth iodide solution) were added. An orange-red precipitate indicates the presence of alkaloids.

#### *3.3.4.3 Test for Terpenoids*

Using Salkowski's test, a liquid chloroformic extract of each sample was prepared by adding 0.5g of the flour extracts into 2 mL chloroform. Concentrated $H_2SO_4$ (3 mL) was added gently down the side of the test tube to form a layer. A reddish-brown ring at the interface indicates the presence of triterpenoids.

### *3.3.4.4 Test for Flavonoids*

Two hundred milligrams of each flour extract were macerated with 20 mL distilled water and filtered. A strip of filter paper was dipped into the extract and allowed to dry. It was then exposed to concentrated ammonia solution, an intense yellow color which disappears when exposed to fumes of concentrated HCl indicates the presence of flavonoids.

### *3.3.4.5 Test for Sterols*

Using the Liebermann-Buchard test, a chloroformic extract was prepared for the powdered flour extract samples and filtered. To 5 mL of the filtrate, acetic anhydride was added followed by concentrated $H_2SO_4$ carefully down the side of the test tube to form a lower layer. A bluish color at the interface indicates the presence of a steroidal ring.

### *3.3.4.6 Test for Phenols*

Two hundreds milligrams of the powdered flour extracts was dissolved in 1 mL of distilled water in a test tube. Two drops of $FeCl_3$ solution was added. A blue, green, red or purple color indicated the presence of phenols.

### *3.3.4.7 Test for Glycosides*

An aqueous solution of the powdered flour extracts were prepared by dissolving about 200 mg of each sample in 1 mL distilled water in a test tube. Few (2) drops of aqueous NaOH were added. The observation of a yellow coloration indicated a positive response for glycosides.

### *3.3.4.8 Test for Cardiac Glycosides*

To 0.5 g of extract diluted to 5 mL in water was added 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.

### *3.3.4.9 Test for Saponins*

To 0.5 g of the flour extracts in a test tube, 5 mL of distilled water was added. The solution was shaken vigorously and observed for a stable persistent froth which indicates the presence of saponins.

## 3.4 Determination of Antinutrients

### 3.4.1 Determination of Oxalate

Oxalate content of the flour samples were determined according to Day and Underwood (1986). About 0.2 gram of each sample was weighed and 40 mL of 1.5N H2SO4 was added. The mixture was stirred intermittently for 1 hour and then filtered with Whatman No 1 filter paper. The filtrate obtained was titrated against while hot against 0.02 M $KMnO_4$ until a faint pink colour was observed which indicated the end point. The oxalate content was calculated as follows;

$$\% \text{ Oxalate Content} = \frac{0.004 \times 0.006303 \times \text{titre value}}{0.02 \times \text{Sample weight}} \times 100$$

### 3.4.2 Determination of Alkaloids Content

Alkaloid content of the flour samples were determined using the method Harborne (1973). Two grams of each sample was weighed into conical flask.

Then 50 mL of 10 % acetic acid in ethanol was added. The mixture was shaken and allowed to stand for 4 hours and filtered using a Whatman No. 2 filter paper. The filtrate was then evaporated for 3mins over a hot plate to about ¼ of its original volume. About 8 mL of (1+1) $NH_4OH$ was added dropwise to precipitate the alkaloids. The precipitate was then filtered off with a preheated and weighed Whatman No. 1 filter paper and washed with 5 mL of 1 % $NH_4OH$ solution. The precipitate in the filter paper was dried in an air oven at 60 °C for 30 minutes and reweighed. The alkaloid content was calculated as follows;

$$\% \text{ Alkaloid Content} = \frac{\text{Difference in weight of filter paper}}{\text{Sample weight}} \times 100$$

### 3.4.3 Determination of Tannin Content

Tannin content was determined according to Fagbemi *et al*. (2005). About 0.2 g of each sample was weighed and 10 mL of 70 % acetone was added. The mixture was shaken for 12-15 minutes to extract the tannins and then filtered. About 2.0 mL of 20 % $Na_2CO_3$ solution was added followed by the addition of 2.5 mL Folin-Ciocalteau and topped up with 70 % acetone. The mixture was incubated at room temperature for 40 minutes. The absorbance readings were taken at 700nm using a spectrophotometer. A calibration curve was drawn with standard tannin acid solutions with concentrations 1ppm, 2ppm, 3ppm, 4ppm and 5ppm. The equation, $y = 0.0669x$ obtained from the standard calibration curve was used for calculating the tannin content in each sample.

### 3.4.4 Determination of Saponin Content

Saponin content was determined according to Nwosu (2011). Two grams (2g) of each sample was weighed and defatted with petroleum ether for 8 hours using the Soxhlet extractor. The defatted samples were further extracted with methanol for 8 hours using pre-heated, cooled and weighed flasks. The extract obtained were dried in the flask on a hot plate, allowed to cool and weighed. The saponin content was calculated as follows;

$$\% \text{ Saponin Content} = \frac{\text{Difference in weight of flask}}{\text{Weight of sample}} \times 100$$

### 3.5 Determination of Beta Carotene

### 3.5.1 Pigment Extraction

Carotene was extracted from the *B. aethiopum* fruit flour samples according to the method by Khalil and Varananis (1996) as described by Ahamad *et al*. (2007). About 2.5g of each flour sample was weighed and homogenized in 50 mL of acetone. The resulting extract was filtered through Buchner's funnel. The residue was washed twice with acetone until it became colorless. The residue was discarded and the filtrate was combined with 20g of anhydrous sodium sulphate. The anhydrous sodium sulphate was removed through filtration and the filtrate (extract) obtained was concentrated to reduce the initial volume 50% using the rotatory evaporator. The extract was transferred quantitatively to 100 mL volumetric flask and diluted to up to the mark with acetone and distilled water, so that the final extract solution was 80% of acetone.

### 3.5.2 Standard Calibration Curve of Beta Carotene and Beta Carotene Determination

Beta carotene and standard calibration curves were done according to Ahamad *et al*. (2007). Five (5) beta carotene standard solutions with concentrations 0.015625, 0.03125, 0.0625, 0.125, and 0.25 µg/g were prepared. Each working standard solution was injected into HPLC system (Agilent 1100 series K15 A306 HPLC 12). Peak identification and quantification was made by "CSW 32 software" for HPLC system. HPLC was calibrated by running mobile phase (hexane and acetonitrile in the ratio 95:5 with 2 drops of triethylamine) at the rate of 2 mL per minute. The wave length was fixed at 452 nm. The pressure of the column was kept 1800-2000 PSI. Twenty microliters (20µL) of each beta carotene standard solution was injected when the injector was in load mode. Concentrations of the beta carotene standards were plotted against the obtained peak areas to obtain a standard calibration curve. The equation of the line was $y=2033.8x + 23.175$ with a correlation coefficient ($R^2$) of 0.996. Twenty microliters (20µL) of beta carotene extract of each flour in 80% acetone was used for HPLC assay like standard; each vegetables sample (20µL) was taken by micro liter syringe. The peaks of the samples were automatically identified and quantified by comparing their retention times with the standard retention time.

### 3.6 Statistical Analysis

The means and standard deviations of all replicated quantifications for each test parameter were calculated using Excel 2013. Using the Statistical Package for Social Sciences (SPSS, IBM SPSS Statistics v20), one-way analysis of variance (ANOVA) was employed to compare the means of all determined parameters. Multiple comparison of all test parameters was carried out using Tukey's test. All statistical tests were carried out 5 % significance level.

# CHAPTER FOUR

## 4.0 Results and Discussion

### 4.1 Mineral Composition of Borassus aethiopum Fruit Flour

Drying had a significant effect on all the determined minerals of the flour samples except sodium. As shown in Appendix 1B, the concentrations of cadmium, copper, lead, manganese, and zinc were below the level of detection (0.002mg/100g). The solar dried samples recorded higher values for all the minerals except for Iron and Sodium. The Calcium and Iron contents of *B. aethiopum* fruit flours in this study as shown in Table 4.1 below are lower than the 107.61-108.25 mg/100g and 2.05-2.15 mg/100g iron respectively in the study of Ali *et al*. (2010a) for the fresh *B. aethiopum* fruit pulp. However, the Magnesium content in this study was higher than the 20.61-21.01 mg/100g for the fresh fruit pulp (Ali *et al*., 2010a). This may be attributed to the difference in soil features as a result of the varying geographical locations

**Table 4.1 Mineral Content (mg/100g) of *Borassus aethiopum* Fruit Flour**

| Mineral | Freeze Dried Flour | Oven Dried Flour | Solar Dried Flour |
|---|---|---|---|
| Potassium | $237.00 \pm 0.39^{a}$ | $268.44 \pm 1.27^{b}$ | $276.73 \pm 2.17^{c}$ |
| Magnesium | $211.61 \pm 11.29^{a}$ | $211.76 \pm 7.66^{a}$ | $293.62 \pm 4.56^{b}$ |
| Iron | $0.60 \pm 0.00^{a}$ | $4.45 \pm 0.02^{b}$ | $0.35 \pm 0.00^{c}$ |
| Calcium | $68.21 \pm 1.35^{a}$ | $80.84 \pm 0.84^{b}$ | $95.92 \pm 0.36^{c}$ |
| Sodium | $28.92 \pm 1.46^{a}$ | $33.38 \pm 1.18^{a}$ | $31.75 \pm 2.47^{a}$ |

*Values are means of replicate determinations. Values in same row with same superscripts are not significantly different (p>0.05). Zinc, Copper, Manganese, Lead (heavy metal) and Cadmium (heavy metal) were all below the level of detection (0.002mg/100g).*

### 4.1.1 Potassium content

The potassium content of the *B. aethiopium* fruit flour ranged from 237.00 to 276.73 mg/100g. There was significant difference among the different drying methods with freeze-drying recording the least value and solar drying recording the highest value. These values were found to be lower than that of *A. altilis flour* (673.5 mg/100 g sample; Appiah *et al*., 2011) but higher than a composite flour made of tigernut flour and HQCF (45.98 to 121.1 mg/100g) and white maize flour (32.80 to 34.90 mg/100g; Shaista Qamar *et al*., 2017). The K content of the freeze dried sample is comparable to that of *Borassus aethiopum* shoots (236.7 mg/100g DW; Umar *et al*., 2002). The values recorded imply that *Borassus aethiopum* fruit flour is relatively good source of potassium and may help in maintaining electrolyte balance in humans (NTBG, 2009).

### 4.1.2 Magnesium Content

The Magnesium content of the solar dried flour was significantly different from the oven dried flour and freeze dried flour. The *Borassus aethiopum* fruit flour irrespective of the different drying methods has higher magnesium content compared to *A. altilis flour* (90.63 mg/100 g sample; Appiah *et al*., 2011), cassava flour (36.58-37.71 mg/100 g; Nassar *et al*., 2003) and flour blends from tigernut flour and HQCF (9.40 to 10.58 mg/100g). Magnesium has been reported to be an essential enzyme system and helps maintain electrical potential in nerves (Ferrao *et al*., 1987), therefore *Borassus aethiopum* Fruit Flour is a very good source of magnesium and can be composited with flours with low magnesium contents due to its high magnesium content.

### 4.1.3 Iron Content

The iron content of the oven dried flour was significantly higher than the freeze dried and solar dried flour. The highest iron content exhibited by the oven dried sample could be as a result of the higher temperature effect of the oven drier thereby increasing the concentration of the iron present in the *Borassus aethiopum* fruit flour. This is corroborated by a study done by Lahav and Turner (2008) where increase in temperature resulted in three-fold increase in iron concentration. The iron content of the flour (0.35-4.45 mg/100g) was lower than cassava flour (32 mg/100g) (FAO and IFAD, 2004) and is also below the recommended daily allowance of iron which is between 8-18 mg /100g as stated by National Academy of Science (2004). Iron is required for the synthesis of hemoglobin and myoglobin, which are oxygen carriers in the blood and muscle respectively. Although the iron content was relatively low, the oven dried flour could help provide some recommended amount of iron in the body when consumed.

### 4.1.4 Calcium Content

The calcium content ranged from 95.92 to 68.21 mg/100g with solar dried flour recording the highest value and freeze drying recoding the least. The calcium level in the flour was higher than that of *A. altilis flour* (52.50-60.83 mg/100 g) reported by Appiah *et al*. (2011) but lower the cassava-tigernut flour blends (138 to 214.3 mg/100g) in a study conducted by Adebowale *et al*. (2016). The relatively high content of the flours in this study indicates it a good source of

calcium and its consumption could help reduce the risk of osteoporosis and provide stronger bones in humans.

### 4.1.5 Sodium Content

The sodium content of the flour was not affected by any of the drying methods thus there was no significance difference among the sodium contents of *B. aethiopum* fruit flour. The sodium content of the flour in this study is comparable to cassava flour (36-50 mg/100g) as reported by Charles *et al*. (2005) but lower than sweet potato (54 mg/100g; Ihekoronye and Ngoddy, 1985) and *A. altilis flour* (69.00 mg/100g). Higher sodium intake is associated with increased risk of hypertension in humans. Also Morgan (1999) stated that reduced intake of low sodium diets ameliorates the development of hypertension. Therefore the low sodium content suggests that *Borassus aethiopum* fruit flour is a low-sodium food and is good for individuals with hypertension.

## 4.2 Anti-nutrient Composition of Borassus aethiopum Fruit Flour

### 4.2.1 Oxalate Content

There was no significant difference among the oxalate contents of the *B. aethiopum* fruit flours. However, the freeze dried flour recorded the highest concentration of 6.47 ± 0.52 g/100g while the oven dried had the least concentration of oxalate of 6.25 ± 0.15 g/100g. The oxalate content of the flour from hypocotyl axes of *Borassus aethiopum* by Ahmed *et al.,* (2010) was relatively lower (0.98 ± 0.05 mg/100g) than the values recorded for this work which is 6.25 ± 0.15 g/100g. The difference in value could be attributed to the fact that different thermal conditions were employed in drying the samples and also

the flours were produced from different parts of the fruits. According to Oguchi *et al.,* (1996) the toxicity level of oxalates is 250 mg/100g but the minimum dose of oxalates that can cause death in adults is 4 to 5 g (Gontzea and Sutzescu, 1968). Oxalates are considered as anti-nutrients due to their ability to chelate and bind to essential minerals such as calcium, magnesium, iron and zinc to form insoluble oxalate compounds that are attached to the gastrointestinal tract and reduces the bioavailability of these nutrients and thus results in mineral deficiency. These insoluble compounds formed calcium oxalates crystals are deposited in the kidney as kidney stones since the body cannot metabolise them. It has been recommended that foods that contains high oxalates content should be consumed with or be accompanied by calcium-rich foods which includes shellfish and dairy products; because most dairy products such as yoghurt contains probiotics which have the ability to digest the deposited insoluble oxalates that are present in the gut (Morrison and Savage, 1999).

**Table 4.2 Anti-nutrient Content of *Borassus aethiopum* Fruit Flour**

| Anti-nutrient | Freeze Dried Flour | Oven Dried Flour | Solar Dried Flour |
|---|---|---|---|
| Oxalate (g/100g) | $6.47 \pm 0.52^{a}$ | $6.25 \pm 0.15^{a}$ | $6.25 \pm 0.60^{a}$ |
| Alkaloid (g/100g) | $0.59 \pm 0.01^{a}$ | $0.22 \pm 0.00^{b}$ | $0.29 \pm 0.01^{c}$ |
| Saponin (g/100g) | $55.62 \pm 0.00^{a}$ | $36.10 \pm 0.00^{b}$ | $42.24 \pm 0.00^{c}$ |
| Tannin (mg/100g) | $23.78 \pm 1.52^{a}$ | $25.28 \pm 2.06^{ab}$ | $31.21 \pm 0.30^{b}$ |

*Values are means of replicate determinations. Values in same row with same superscripts are not significantly different ($p>0.05$).*

### 4.2.2 Alkaloid Content

Results obtained for the phytochemical screening as shown in Table 4.4 indicate the absence of alkaloids in *B. aethiopum* fruit flour. However, Table 4.2 shows the alkaloids content of the flours ranged from 0.22 ± 0.00 g/100g for the oven dried flour to 0.59 ± 0.01 g/100g in the freeze dried flour. These values were significantly different from each other at p=0.05. Although, alkaloids concentrations were the least among all determined anti-nutrients content of *B. aethiopum* fruit flour, its concentrations exceeded the 20 mg/100g reported by Inuwa *et al*. (2011). Alkaloids are considered to be anti-nutrients because of their actions and how they affect the nervous system, disrupting and increasing of electrochemical transmission. At high concentration of alkaloids, it can cause rapid heartbeat, paralysis and in lethal dose can result in death. Alkaloids also cause the disruption of the cell membrane found in the gastrointestinal tract (Fernando *et al.*, 2012; Gemede and Ratta, 2014).

### 4.2.3 Saponin Content

Drying method had a significant effect on the saponin content of *B. aethiopum* fruit flours. Saponins were the highest in value among the determined anti-nutrients. Oven dried flour had the least saponin content (36.10 ± 0.00 g/100g) while freeze dried flour had the highest value of saponins (55.62 ± 0.00 g/100g). All the saponin values recorded are significantly different from each other. It has been reported that saponins has a list of biological activities such as being antiobesity, antioxidant, antiparasitic, antiulcer and diuretic among others. According to Toyin *et al*. (2012), saponin has anti-diarrhoeal effects hence its

presence in *B. aethiopum* fruit flour gives it some medicinal benefits. Adversely, saponins at high concentrations above the tolerable limits have negative effects on the absorption of minerals and vitamins, cognitive behaviour, ethanol induced amnesia and the inhibition of active nutrient, such as protein, transportation in the body (Hostettmann and Marston, 1995; Lacaille-Dubois and Wagner, 1996; Francis *et al.*, 2002). Saponin is regarded as an anti-nutrient because of its harmful effects on growth impairment and reduce their bitterness and throat-irritating activity that reduces their intake. They decrease the bioavailability of nutrients, decrease enzyme activity and affect protein digestibility by inhibiting some digestive enzymes such as trypsin and chymotrypsin which can lead to protein deficiency (Liener, 2003). However, the toxicity of saponin to warm blooded mammals like humans depends on the manner at which it is administrated, its source, concentration and composition of the saponin content (George, 1965; Oakenfull and Sidhu, 1990). At low concentrations, saponins has hypocholesterolemic, immunostimulatory and anticarcinogenic properties (Oakenfull and Sidhu, 1990; Liener, 2003).

### 4.2.4 Tannin Content

Tannins is an astringent, bitter plant polyphenolic compound that either binds or precipitates proteins and various other organic compounds including amino acids and alkaloids (Redden *et al.*, 2005). Table 4.4 showed that tannins were present in the *Borassus aethiopum* fruit flours. There was a significant difference between the tannin contents of the freeze dried and solar dried flours. However, the concentration of tannins in the solar dried flour recorded the highest value of

31.21 ± 0.30 mg/100g while tannins concentration in the freeze dried flour was the least with the value of 23.78 ± 1.52 mg/100g. Although the tannin content of *B. aethiopum* fruit flours were higher than the 3mg/100g lethal dose stated in the work of Inuwa *et al*. (2011) the tannin contents of the fruit flours are lower than the 239.76 mg/100g reported by Ahmed *et al.* (2010) for the hypocotyl axes flour. According to Ali *et al*. (2010b) and Gbesso *et al*. (2016), the hypocotyl axes has been a food supplement for some rural Africans in Togo, Cameroon and Nigeria. Medicinally, tannins are used as haemostatic, antidiarrheoal, antiheamorrhoidal and anti-inflammatory agents (Cheng *et al.,* 2002). The high tannin content of *B. aethiopum* fruit flour makes it applicable in the pharmaceutical as Amabeoku (2009) suggested for plants with tannin contents.

## 4.3 Antioxidant Properties and Beta-Carotene Content of B. aethiopum Fruit Flour

### 4.3.1 Total Phenol Content

The total phenolic content (TPC) of African palmyra palm fruit flour ranged from 1518.00 mg GAE/100g in solar dried flour to 3896.71 mg GAE/100g in the freeze dried flour samples as shown in Table 4.3. There was a significant difference between total phenolic content of freeze dried flour samples and solar dried flour samples. However, there was no significant difference between oven dried flour samples and both freeze dried and solar dried flour samples.

Drying at lower temperatures retains more heat labile phytochemicals including phenols than drying at higher temperatures (Sim *et al*., 2017). Hence the higher total phenol content in the freeze dried flour. The finding in this study is contrary

to that of Dossou *et al*. (2014) who reported that freeze dried ackee fruit arils retained less phenols compared to oven dried ackee fruit arils. This could be attributed to differences in the types of phenols in the various plant materials. The lower total phenol content of solar dried flour could be due to increased oxidative reactions during the drying period (Zanoni *et al*., 1999; Zanoni *et al*., 2000; Toor and Savage, 2006) which may lead to the loss of phenols and other antioxidants via reaction with oxidants.

The high total phenol content of the flour could be responsible for its relatively high free DPPH scavenging activity (IC50 from 2.05 – 4.14 mg/mL). Phenols exhibit free radical scavenging activities thus have the ability to minimize the oxidative breakdown of biomolecules (Ozgova *et al*., 2003; Kang *et al*., 2005; Amoateng *et al*., 2010). Scalbert and Williamson (2000) recommended consumption of 1000 mg GAE/day of phenols hence consuming 100g of *B. aethiopum* a day could meet this nutritional requirement.

**Table 4.3 Total Phenolic Content, IC50 and Beta-Carotene Content of *B. aethiopum* Fruit Flour**

| Parameter | Freeze Dried Flour | Oven Dried Flour | Solar Dried Flour |
|---|---|---|---|
| TPC (mg GAE/100g) | $3896.71 \pm 774.61^{a}$ | $2550.86 \pm 66.40^{ab}$ | $1518.00 \pm 509.03^{b}$ |
| IC50 (mg/mL) | $2.05 \pm 0.00^{a}$ | $2.55 \pm 0.29^{a}$ | $4.14 \pm 0.05^{b}$ |
| Beta-Carotene(μg/100g) | $3.36 \pm 0.00^{a}$ | $4.19 \pm 0.79^{a}$ | $2.06 \pm 0.89^{a}$ |

*Values are means of replicate determinations. Values in same row with same superscripts are not significantly different (p>0.05).*

### 4.3.2 DPPH Scavenging Activity

The results for DPPH activity of the *B. aethiopum* flour are as shown in Figure 4.1 below and in Table 4.3 above. IC50 values (in Table 4.3) ranged from 2.05 mg/mL in freeze dried flour to 4.14 mg/mL in solar dried flour. The IC50 for African palmyra palm fruit flour in this study were higher than the 1.20 mg/mL reported for the fresh pulp by Amoateng *et al*. (2010).

There was no significant difference between the IC50 values for both the freeze dried and oven dried flours. IC50 values indicate antioxidant activity which also depends the total phenols and other antioxidants present. As shown in figures 4.2 and 4.3, strong positive correlations existed between the total phenol contents and IC50 and also between the beta-carotene content and the IC50 values of the flours.

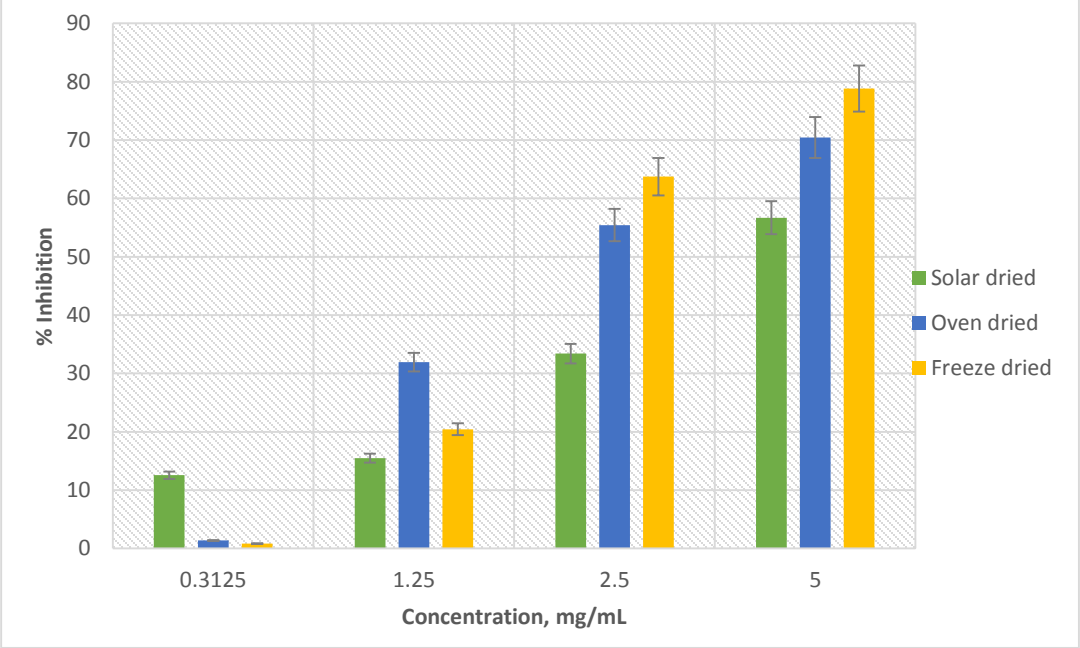



**Figure 4.1. Antioxidant Activity (% DPPH inhibition) at Different Flour Extract Concentrations**

*B. aethiopum* fruit flour showed a concentration dependent DPPH inhibition activity as shown in Fig 4.1. The higher the concentration of the flour extract, the higher its free radical scavenging ability.

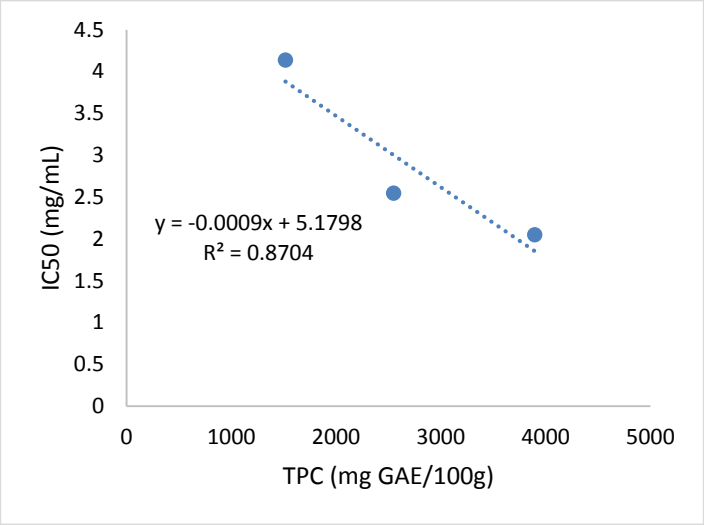



Fig. 4.2 Correlation between total phenol content and IC50 of *B. aethiopum* fruit flour

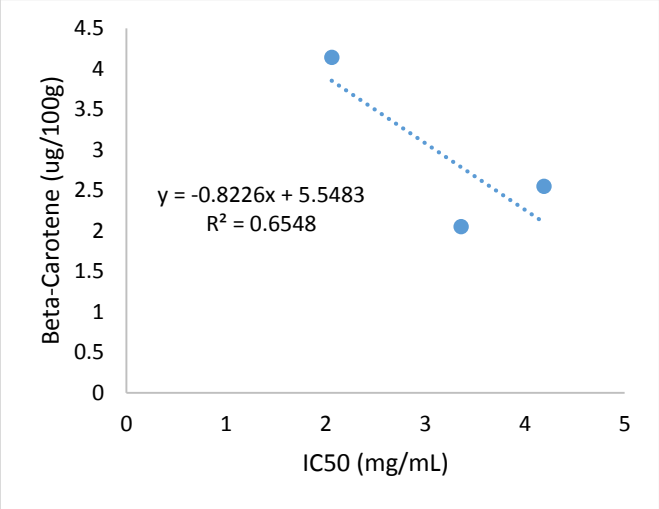



Fig. 4.3 Correlation between beta-carotene content and IC50 of *B. aethiopum* fruit flour

### 4.3.3 Beta-Carotene Content

The beta-carotene content of *B. aethiopum* fruit flour ranged from 2.06 µg/100g in the solar dried flour to 4.19 µg/100g in the oven dried flour. However, there difference among the beta-carotene content for all the flours was not significant. The low beta-carotene content could be influenced by the drying conditions. According to Ali *et al*. (2010c), oven drying at 40°C for 48 h decreased the total carotenoid content of *B. aethiopum* fruit pulp by 37 – 40 %. Drying is said to decrease the concentration of phytochemicals including antioxidants via browning reactions which are increased in the presence of moisture, heat and air (Zanoni *et al*., 2000; Toor and Savage, 2006; Wiriya *et al*., 2009). The double bonds present

in the chemical structure of beta-carotene makes it susceptible to thermal degradation. Beta-carotene is the precursor for vitamin A hence its presence in food materials from plant sources is desirable.

## 4.4 Phytochemical Screening of *Borassus aethiopum* Fruit Flour

As shown in Table 4.4 below, the phytochemical tests indicated the presence of tannins, saponins, glycosides, phenols, sterols, terpenoids, cardiac glycosides and the absence of flavonoids and alkaloids in the methanolic extracts of all the flour samples. However, Table 4.2 above indicated the flour samples had 0.22 – 0.59 % alkaloids. The phytochemical composition of the *B. aethiopum* fruit flour was similar to the finding of Sarkodie *et al*. (2015) who reported the presence of tannins, phenols, saponins, triterpenoids and alkaloids for the ethanolic extract of the fresh fruit pulp in their study.

Alkaloids, terpenoids, glycosides, saponins, sterols, and tannins been reported to have anti-diarrhoeal effects due to their anti-enteropooling effects via the inhibition of the release of autocoids and prostaglandins (Galvez *et al*., 1993; Havagiray *et al*., 2004; Perez *et al*., 2005, Tiwari *et al*., 2011; Toyin *et al*., 2012).

Phytochemicals also have antioxidant properties, anti-inflammatory and antibacterial effects hence their use in treatment of diseases and bacterial infections. Sarkodie *et al*. (2015) reported that fresh ethanolic extract of *B. aethiopum* fruit pulp had anti-inflammatory and anti-microbial effects which were attributed to the phytochemical composition of the extract. Therefore, the

phytochemical composition of *B. aethiopum* fruit flour shows its potential medicinal benefits.

**Table 4.4 Phytochemical Screening of *Borassus aethiopum* Fruit Flour Extract**

| Test | Solar Dried Flour | Freeze Dried Flour | Oven dried Flour |
|---|---|---|---|
| Tannins | + | + | + |
| Saponins | + | + | + |
| Alkaloids | - | - | - |
| Glycosides | + | + | + |
| Phenols | + | + | + |
| Sterols | + | + | + |
| Terpenoids | + | + | + |
| Flavonoids | - | - | - |
| CG (kedde's test) | + | + | + |
| CG (keller-kiliani's test) | + | - | - |

+ = *Present;* - = *Absent*; CG = Cardiac glycosides

Although saponins and tannins are anti-nutrients, they also function as antioxidants in the body when consumed at their acceptable levels. The negative test response for alkaloids and flavonoids could be due to their insolubility in methanol which was used in this study for the flour extract preparation. According to Cowan (1999) alkaloids and flavonoids are soluble in ethanol and chloroform respectively hence could only be extracted with these solvents.

# CHAPTER FIVE

## 5.0 Conclusion and Recommendations

### 5.1 Conclusion

*Borassus aethiopum* fruit flour had relatively high levels of potassium, magnesium; considerable amount of sodium, calcium and very low iron content. However, lead, cadmium and zinc concentrations *B. aethiopum* fruit flour were below detection limits. *Borassus aethiopum* fruit flour could be a good source of potassium and magnesium. The flour also had high levels of total phenols as well as considerable free radical scavenging activities. *B. aethiopum* fruit flour however had low levels of beta-carotene and tannins but relatively high levels of saponins and oxalates. The presence of tannins, phenols, sterols, terpenoids, glycosides and saponins gives *B. aethiopum* fruit flour potential medicinal applications as anti-diarrhoeal, anti-microbial and anti-inflammatory agents.

Drying had a significant effect on all the detected minerals except sodium, total phenol content, DPPH scavenging activity, cardiac glycosides, saponin content, tannin content and alkaloid content of *B. aethiopum* fruit flour. However, there was no significant variation among the oxalate and beta-carotene contents of the flour.

### 5.2 Recommendations

- The phytochemical constituents and antioxidant properties of the *Borassus aethiopum* fruit flours should be investigated using different extracts including chloroformic, ethanolic and aqueous extracts.

- The glycosides, phenols, sterols and triterpenoids contents of *Borassus aethiopum* fruit flour should be determined.

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# APPENDICES

## Appendix 1A. Images of *Borassus aethiopum* fruit flour


Fig.1 Freeze Dried Flour


Fig.2 Oven Dried Flour

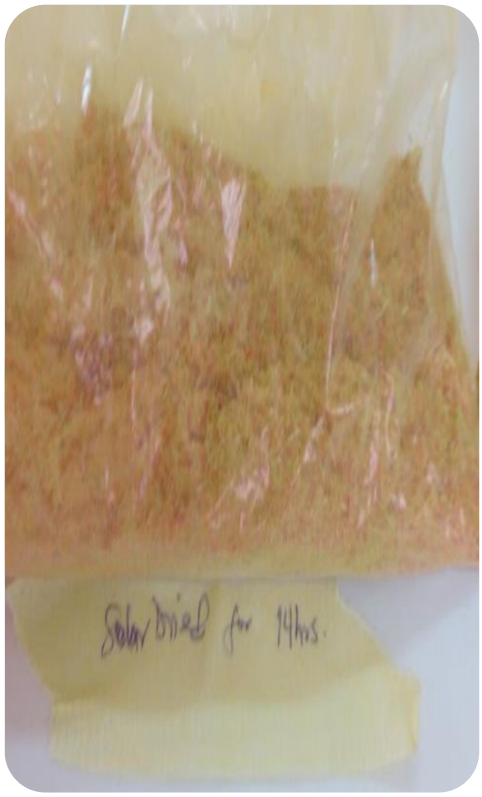


Fig.3 Solar Dried Flour

**Appendix 1B. Mineral Composition of *B. aethiopum* Fruit Flour in mg/100g**

| Sample | K | Mg | Fe | Na | Ca | Zn | Cd | Pb | Cu | Mn |
|---|---|---|---|---|---|---|---|---|---|---|
| F1 | 236.735 | 203.630 | 0.596 | 27.882 | 69.166 | BDL | BDL | BDL | BDL | BDL |
| F2 | 237.281 | 219.595 | 0.594 | 29.948 | 67.250 | BDL | BDL | BDL | BDL | BDL |
| O1 | 269.338 | 217.176 | 4.443 | 32.547 | 81.436 | BDL | BDL | BDL | BDL | BDL |
| O2 | 267.544 | 206.340 | 4.466 | 34.211 | 80.243 | BDL | BDL | BDL | BDL | BDL |
| S1 | 278.266 | 296.844 | 0.348 | 30.004 | 95.665 | BDL | BDL | BDL | BDL | BDL |
| S2 | 275.200 | 290.400 | 0.348 | 33.500 | 96.180 | BDL | BDL | BDL | BDL | BDL |

**Appendix 1C. Phytochemical Screening Results for *B. aethiopum* Fruit Flour.**

| **Test** | **Sample** | | |
|---|---|---|---|
| | **Solar dried** | **Freeze dried** | **Oven dried** |
| Tannins | Positive | Positive | Positive |
| Saponins | Positive | Positive | Positive |
| Alkaloids | Negative | Negative | Negative |
| Glycosides | Positive | Positive | Positive |
| Phenols | Positive | Positive | Positive |
| Sterols | Positive | Positive | Positive |
| Triterpenoids | Positive | Positive | Positive |
| Flavonoids | Negative | Negative | Negative |
| Cardiac (kedde's) | Positive | Positive | Positive (Purple) |
| Cardiac (keller-kiliani's) | Positive | Reddish brown ring observed (Negative) | Brown ring (Negative) |

## APPENDIX 2.0 ANOVA Tables for Mineral Composition, Antioxidant Properties and Anti-nutrient Composition of *B. aethiopum* Fruit Flour

### Appendix 2.1 ANOVA Table for Mineral Composition of *B. aethiopum* Fruit Flour

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Potassium | Between Groups | 1756.578 | 2 | 878.289 | 407.972 | .000 |
| | Within Groups | 6.458 | 3 | 2.153 | | |
| | Total | 1763.036 | 5 | | | |
| Magnesium | Between Groups | 8951.529 | 2 | 4475.765 | 64.894 | .003 |
| | Within Groups | 206.913 | 3 | 68.971 | | |
| | Total | 9158.442 | 5 | | | |
| Iron | Between Groups | 21.213 | 2 | 10.607 | 119399.968 | .000 |
| | Within Groups | .000 | 3 | .000 | | |
| | Total | 21.214 | 5 | | | |
| Sodium | Between Groups | 20.415 | 2 | 10.208 | 3.180 | .181 |
| | Within Groups | 9.630 | 3 | 3.210 | | |
| | Total | 30.045 | 5 | | | |
| Calcium | Between Groups | 770.097 | 2 | 385.048 | 431.062 | .000 |
| | Within Groups | 2.680 | 3 | .893 | | |
| | Total | 772.777 | 5 | | | |

### Appendix 2.2 ANOVA Table for Antioxidant properties and Beta-Carotene Content of *B. aethiopum* Fruit Flour

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| IC50 | Between Groups | 4.756 | 2 | 2.378 | 84.717 | .002 |
| | Within Groups | .084 | 3 | .028 | | |
| | Total | 4.840 | 5 | | | |
| TPC | Between Groups | 5690948.604 | 2 | 2845474.302 | 9.885 | .048 |
| | Within Groups | 863535.732 | 3 | 287845.244 | | |
| | Total | 6554484.337 | 5 | | | |
| Beta-carotene | Between Groups | 4.580 | 2 | 2.290 | 4.821 | .116 |
| | Within Groups | 1.425 | 3 | .475 | | |
| | Total | 6.005 | 5 | | | |

**Appendix 2.3 ANOVA Table for Anti-nutrient Composition of *Borassus aethiopum* Fruit Flour**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Oxalate | Between Groups | .068 | 2 | .034 | .154 | .864 |
| | Within Groups | .657 | 3 | .219 | | |
| | Total | .725 | 5 | | | |
| Alkaloid | Between Groups | .152 | 2 | .076 | 2274.500 | .000 |
| | Within Groups | .000 | 3 | .000 | | |
| | Total | .152 | 5 | | | |
| Saponin | Between Groups | 422.910 | 2 | 211.455 | 12687309.000 | .000 |
| | Within Groups | .000 | 3 | .000 | | |
| | Total | 422.910 | 5 | | | |
| Tannin | Between Groups | 61.717 | 2 | 30.859 | 13.886 | .030 |
| | Within Groups | 6.667 | 3 | 2.222 | | |
| | Total | 68.384 | 5 | | | |