KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

The Anti-Lipidemic Effect of *Borassus aethiopum* 'Oman Kube' on Individuals with Cardiovascular Disease at the 37 Military Hospital in Accra, Ghana

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By

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DECLARATION

I declare that I have wholly undertaken the study and reported herein under the supervision of Dr. Charles Apprey and that except portions where references have been duly cited, this dissertation is the outcome of my research.

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ABSTRACT

There is a rise in the prevalence of cardiovascular disease (CVD) globally and it is expected that by 2030, related death associated with CVD will be about 23.6 million mainly from heart disease and stroke. Obesity and lifestyle changes have been attributed as the main causes. Obesity associated with dyslipidemia is one of the factors known to initiate atherogenesis in CVD progression. A single blinded randomized placebo-controlled trail was conducted on 122 CVD patients at the Medical Department of the 37 Military Hospital in Accra, Ghana. The objective of the study was to evaluate the anti-lipidemic effect of *Borassus aethiopum* among individuals with CVDs. The *Borassus aethiopum* pulp was used as composite flour for bread. Sensory analysis, phytochemicals present and microbial analysis was conducted on the composite bread. About 150g dough weight of bread was consumed by participants daily for three months. Participants were randomized into two groups. Baseline and post interventional BMI, visceral fat, body fat, waist circumference, blood pressure and serum levels of total cholesterol (TC), triglycerides, high density lipoprotein (HDL-C), low-density lipoproteins (LDL-C) were determined. Data was analysed using IBM SPSS version 23. The *Borassus* composite bread contained flavonoids, saponins, phenols, alkaloids, glycosides, sterols and steroids with microbes within accepted levels (1.75×10^3 ±7.07 cfu/g for flour and 2.31×10^3 ±7.07 cfu/g for the bread). Participants recruited included, 63.9% female and 36.1% male, age ranging from 19 to 70 years. Most (99.2%) were diagnosed of hypertension, dyslipidemia or both. Diabetes mellitus (7.4%), and stroke (1.6%) with 37.7% on lipid lowering medication. Some female 53.3% and some male 7.3% were centrally obese. Baseline systolic blood pressure above normal was 73.8% and diastolic 81.9%, reducing significantly after intervention ($p = 0.019, 0.001$) respectively. Using the NCEP ATP 111 (2002) guidelines, significant reduction was observed in participants total cholesterol above the normal range and between groups ($p=0.002$). At baseline, the mean results for LDL-C was 3.4±1.2 for females and 3.3±1.3 for males. These saw some significant reduction among those who were given the composite bread ($p=0.0002$). Even though 90.1% of participant's TG and (female-97.6% and male-94.8%) HDL-C recorded normal levels, reductions in their means were also recorded. Among female participants, strong association existed only between their TC and LDL-C (r-0.930; $p=0.000$) and TC and HDL-C ($r=0.460$ $p= 0.000$). Weaker associations were realised between some other parameters. Similar associations were realised within parameters male participants. Strong correlations existed between Systolic and diastolic (r-0.725 $p=0.000$), TC and LDL-C (r-0.900 $p=0.000$) and TC and HDL-C (r-0.526 $p=0.000$). Among those on medication, significant reductions were realised in systolic ($p=0.005$), diastolic (p-value=0.0003), LDL-C ($p=0.048$) and HDL-C ($p=0.00039$) after intervention. With respect to their anthropometries, with the exception of waist circumference, no significant difference was seen in participant on the various medications. *Borassus aethiopum* composite bread was seen to reduce appreciably the TC, LDL-C and WC of cardiovascular patients in the study, irrespective of the medication and dosage participant were on.

LIST OF ABBREVIATIONS

CVD- Cardio Vascular Disease

ROS – Reactive Oxygen Species

CHD- Chronic Heart Disease

WHO- World Health Organization

NO – Nitric Oxide

LDL – Low Density Lipoproteins

WC - Waist Circumference

BMI - Body Mass Index

TC - Total Cholesterol

TG – Triglyceride

HDL-C - High Density Lipoprotein- Cholesterol

NHMRC - National Health and Medical Research Council

mmol/L - Millimole per Liter

BP - Blood Pressure

mmHg - Millimeter per Mercury

LMIC – Low - Middle Income Countries

WHF- World Heart Federation

AHA- American Heart Association

CHD- Chronic Heart Disease

NEFA – Non Esterified Fatty Acid

CETP – Cholesteryl Ester Transfer Proteins

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CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND OF STUDY

Cardiovascular diseases (CVDs) are conditions resulting from disorders or malfunctioning of the heart and blood vessels. This includes hypertension, coronary heart disease, cerebrovascular accidents (stroke), rheumatic heart disease and others (WHO, 2015). Non-communicable diseases particularly cardiovascular disease remain an area of high public health concern affecting both male and female of varied ages with risk proportional to age. Regardless of the numerous research in this area, with various approaches, prevalence is still high. It is rated the leading cause of death globally, with an increased population dying annually from the disease than from other conditions (WHO, 2015). About 17.3 million people were estimated to die from the condition in 2008, this represented 30% of all estimated deaths globally. Of these deaths, 7.3 million were again estimated to be due to coronary heart disease and 6.2 million, stroke (WHO, 2015). Undoubtedly, middle- and low-income countries recorded over 80% of all CVD deaths mainly from heart disease and stroke. Out of five CVD deaths, four are due to heart attacks and strokes. Stroke mortality-to-incidence ratio (MIR) for 2010 was also high. In Africa and Sub-Saharan African countries, the burden of cardiovascular disease has seen a steady increase, with CVD-related deaths accounting for almost 9.2% of all deaths (Roth *et al.,* 2015). Among those aged over 45 years, CVD is the leading cause of death (Livesay, 2007; Mocumbi, 2012). In 2010, the leading CVD cause of death and mortality in Africa was stroke. Again in 2012, nearly half a million deaths due to stroke was recorded in Sub-Saharan Africa representing 4.4% of all deaths in the region (WHF, 2015). In Ghana like most African countries, the situation is the same. According to the WHO, (2014) Coronary Heart Disease Deaths in Ghana was 6.5% of total deaths. The age adjusted Death Rate was 96.7 per 100,000 of the population, ranking Ghana the 75th in the world (WHO, 2015). Regarding the top fifteen diseases in Ghana, stroke ranked first, coronary heart disease third and hypertension

twelfth (WHO, 2015). In as much as causes of CVD are multifaceted, over the years, atherosclerosis was regarded the primary and passive process of accumulating cholesterol but Appannah *et al.* (2015), indicated that, atherosclerosis is an active process involving components of the blood vessels, immune, metabolic and endocrine systems. Until recently, the immune- biochemical pathways activated in the process of atherosclerosis, have led to oxidative stress-related pathological consequences strongly linked again to CVDs. Overwhelmingly, the stress caused by oxidation in the body is critical, not only because it triggers immune responses but also because it leads to endothelial and smooth muscle dysfunction, the progression of atherosclerosis (Griendling and FitzGerald, 2003; Galle *et al*., 2006; Dzau *et al.*, 2006). In addition to oxidation of LDL's, changing lifestyle practices, poverty, urbanization and globalization also play a role in disease initiation and progression. Irrespective of the fact that heredity contributes to increase in prevalence, raised blood pressure, blood glucose, dyslipidemia, smoking and obesity have been identified by Asfaw (2005), to be some of the risk factors associated to CVD's. Currently, advances in the knowledge of both the disease processes and healthy dietary components have been documented, providing new ways of developing pharmaceutical and or dietary strategies to control or reduce the development of these vascular diseases. Kizhakekuttu and Widlansky (2010), and Dauchet *et al.* (2006), noted that, several foods containing antioxidant vitamins and flavonoids are strongly associated with a decrease in oxidative stress and its related diseases.

Borassus aethiopum commonly known as the African Fan Palm or African Palmyra Palm is a tropical plant species widely found across sub-Saharan Africa countries. It is a widespread wild plant found in most West African countries such as Ghana, Nigeria, Togo and Guinea and belongs to family *Arecaceae* (Bolade and Bello, 2006; Small, 2011). Globally, it is noted for its medicinal and non-medicinal purposes. In Ghana, like other African countries, it is used as food and for other purposes and found in various regions; particularly in the Ashanti, northern and Volta regions. *Borassus aethiopum* called 'oman kube' by the Ashanti's is found in the 'Abrimasu' forest reserve of Mampong forest

District in the Ashanti region of Ghana (Kobreti, Kwaseakan, Teacherkrom, Adome and Aframso). In the Volta region, the palm is called "agoteku' and can be found in Adaklu, North Tongue, Kpetoe-Ziope and Akatsi North. The fruits are often consumed as food or in the form of food supplements (Ali *et al*., 2010a; Agbo N'zi *et al.,* 1992). Nutritionally, the pulp of the juicy matured fruit has an orange to yellow fibrous tissue rich in sugar, vitamin A, B and C (Nilugin and Mahendran, 2010). It is also known to possess a variety of phytochemicals, anti-inflammatory and anti-oxidant properties (Kapoor, 2000). The phytochemical proven to be present are alkaloids, tannins, saponins, triterpenes and phenols with a total antioxidant capacity of 329.4 mg of ascorbic acid equivalent per gram (Sarkodie *et al.,* 2015). Despite its rich source of phytochemicals, it is relatively cheap and under-utilized by most Ghanaians. These rich phytochemical constituents render it a good potential in the management of cardio vascular related diseases. Preventive measures as well as available foods with medicinal properties can be used in the treatment and or management of such conditions. This will go a long way to reduce the burden on the country and escalating rate of CVD's. Despite the fact that some research has already been done using hamsters in the evaluating the anti-diabetic properties of the *Borassus aethiopum* fruit pulp (Larbi *et al.,* 2016), much has not been done using the *Borassus aethiopum* to evaluate its anti-lipidemic properties. In view of this there is therefore the need for some further research to be conducted particularly in humans; hence the objective of this work was to examine the effect of *Borassus aethiopum* on the lipid profile of some individuals with cardiovascular disease.

1.2. PROBLEM STATEMENT

Non communicable diseases particularly cardiovascular diseases have become a public health issues worldwide. It is rated the leading cause of death, with an estimated increase in the death toll as against other conditions (WHO, 2015). In 2008, 17.3 million people were estimated to die from the condition and this represented 30% of all estimated deaths globally. Most of these deaths were undoubtedly

reported in the middle- and low-income countries. In Ghana like most African countries, CVD related deaths are high (WHO, 2015). Regardless of the increase in research in this area and various approaches, prevalence is still high. These high rates may probably be due to multifaceted causes and risks factors including globalization, obesity, life style changes, inactivity and smoking (Asfaw, 2005). These factors result in oxidative stress known to initiate atherosclerosis, a major mechanism responsible for CVD initiation and progression. However, irrespective of the cause of the oxidative stress, several other foods containing antioxidant vitamins and flavonoids have been strongly associated with a decrease in CVD and its related diseases (Dauchet, *et al.,* 2006; Kizhakekuttu & Widlansky, 2010).

Borassus aethiopum, a local plant may be used to alleviate oxidative stress hence CVD's. The pulp of the fruit is noted to contain several phytochemicals; flavonoids, alkaloids, triterpenes, cardiac glycosides, sterols and steroids as well as anti- oxidants and anti- inflammatory properties in appreciable amounts (Gruca *et al.,* 2015; Amoateng *et al.,* 2010). Some research has been done already using the fruit to evaluate the impact of its phytochemicals on hamsters induced with hyperglycaemia and its anti- plasmodia effect in humans (Gruca *et al*., 2015; Larbi *et al.,* 2016). In Ghana, even though the fruit is abundant, known and inexpensive, its use is limited. Irrespective of the amount of nutrients and phytochemical present, its bitter taste, process of peeling and making it ready as food is difficult. The tough and hard nature of pericarp and seed is one of the reasons for this, often leaving the fruit to rot under these trees when ripe. To overcome the challenges mentioned before, it would be necessary to explore ways of introducing *Borassus aethiopum* into bread to assist in reducing oxidative stress-related diseases hence CVDs.

1.3. AIM OF STUDY

The objective of this study was to evaluate the impact of *Borassus aethiopum*-composite bread in lowering lipid levels of individuals with cardiovascular diseases (CVDs).

1.4. SPECIFIC OBJECTIVES

1. To assess the gender and age range most affected by dyslipidemia and their state of dyslipidemia
2. To determine the phytochemical composition, phenolic content and anti-oxidant activity of *Borassus aethiopum* fruit pulp (powder)
3. To investigate the use of *Borassus aethiopum*-composite flour in bread making
4. To assess the effect of *Borassus aethiopum* composite bread in lowering blood lipid in individuals with CVDs.

1. 5. SIGNIFICANCE OF THE STUDY

1- The findings of this study will be useful in the health sector; inferences from the study finding will be used in managing CVDs.

2- Dissemination of outcome of the research to policy makers, would aid in the formation of protocols to be used by dieticians and medical professionals in management of cardiovascular diseases.

3- Another healthy product will also be available for use by patient with CVD as well as the general public.

4- Findings of this study will add to the body of knowledge and facilitate future research into bread phytochemical blend for managing other disease conditions

1.6. RESEARCH QUESTION

1. What are the phytochemicals found in *Borassus aethiopum*?
2. Can *Borassus aethiopum* be used as composite flour for making other products (bread)?
3. What is/are the effect of *Borassus aethiopum* on the lipids lowering capacity of individuals with CVDs?

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. INTRODUCTION

Data was collected from several databases such as such as science direct, sage, medline, CINHAL, google scholar, AHFS Consumer Medication Information, Health Source - Consumer Edition and ebscohost. Key words such as "proximate and phytochemical composition of *Borassus aethiopum* fruit (pulp)", "uses of *Borassus aethiopum*" and "antioxidants from *Borassus aethiopum* and their effect on lowering blood lipid (cholesterol) in hyperlipidemics", phytochemical properties of *Borassus aethiopum,* pathophysiology of cardiovascular disease, and causes of cardiovascular disease were entered into the search button to generate the desired results based on the objectives. Review of the literature started in 2016 and articles reviewed were narrowed to the scope of the study. Finally, some articles which were within the scope of the study were used for the review ranging from 2010 to 2016. The gaps after the review of the literature were clearly identified and efforts were to fill that gap in the present study.

2.2. CARDIOVASCULAR DISEASES

Cardiovascular diseases (CVDs) are one of the most common non-communicable diseases resulting from disorders or malfunctioning of the heart and blood vessels at both micro and macro levels. Hypertension, coronary heart disease, cerebrovascular accidents (stroke), rheumatic heart disease and others are all included in this category (AHA, 2012 and WHO, 2015).

Rolfes *et al*., (2009) describe CVDs as a group of diseases that halt or impair the normal functions of the heart and its related blood vessels. Ideal, intermediate and poor cardiovascular health is based on the basic risk factors such as smoking, increased body mass index, physical inactivity, unhealthy diet, dyslipidemia, elevated blood pressure, and fasting plasma glucose (Lloyd-Jones *et al.*, 2010; Martin-

Timon *et al.*, 2014). The risk factors associated with CVD is similar globally, high blood pressure, obesity, physical inactivity, abnormalities in lipid profile, smoking and reduced consumption of fruits and vegetables (Micah and Nkum, 2012). Additionally, family history of CVD, particularly first degree relative, has been noted to increase risk 1.5 times and double if more than one first degree relative has such a history (Wood *et al.*, 2005).

2.3 PREVALENCE OF CARDIOVASCULAR DISEASE

2.3.1 Prevalence of Cardiovascular diseases (CVDs)

Regarded as the number one cause of death globally, in 2012, 17.5 million people estimated deaths were due to CVD and these deaths increased in 2015 worldwide. Of these deaths, an estimated 7.4 million and 6.7 million were due to chronic heart disease (CAD) and stroke, respectively. Undoubtedly, majority (87%) of these deaths occurred in low and middle-income countries (LMIC) (WHF, 2015; WHO, 2015). In 2012, nearly half a million deaths due to stroke was recorded in Sub-Saharan Africa representing 4.4% of all deaths in the region (World Health Federation, 2015). Furthermore, in 2010, Africa recorded stroke as the leading cause of death and mortality. In Ghana like most African and sub-African countries, the situation is the same. According to the WHO (2014) coronary heart disease deaths in Ghana was 6.48% of total deaths. In Ghana, with regards to the top first fifteen diseases published by the world health organization, the story was not different; stroke ranked first, coronary heart disease third and hypertension twelfth (WHO 2015).

2.3.2. Prevalence of Causes and Risk Factors

The causes and risk factors of CVDs also increase in prevalence yearly. The World Health Organization predicted a doubling of prevalence of obesity (BMI $\geq 30 kg/m^2$) globally, from 1998-2008. Chow *et al.* (2008), also confirmed this when he observed the steady increase in obese participants. He

reported an increase of 137% for overweight and 88% for hypercholesterolemia among Indians, of which, 95% were men and 65% were women. Other studies have shown high blood pressure, another risk factor to be on the ascendency, a rise of 400 million people from 1980 -2008 (WHO, 2014). Individuals aged 25 years and above representing 40%, were mostly from low – middle income countries (LMIC). In Ghana, Addo *et al.* (2012), also identified hypertension both diagnosed and pre-diagnosed and dyslipidaemia to be on the increase within the same age group of people.

2.4. ECONOMIC BURDEN OF CARDIOVASCULAR DISEASES (CVDs)

The burden of CVDs on a nation is proportional to its prevalence; currently gaining more public concerns in most endemic countries. Generally, the disability adjusted life years (DALYS) for low-middle income countries (LMIC) is about 10% as compared to 18% for high income countries (HIC). In addition, the total years of life lost (YLL) is about four times higher in LMIC than HIC (WHF, 2015). It is also estimated that, an increase of 61% from 171.7 to 275.8 billion dollars would be spent as indirect cost by these countries between 2010 and 2030 (Mathers *et al*., 2016). Even though the WHO, has predicted that, economic loss on CVD will be higher than most non-communicable diseases, (Figure 2.1) economist have also predicted that in the next 25 years, the cost of not investing in the prevention and treatment of CVD will rise to as high as 47 trillion dollars obviously with developing countries experiencing more consequences than developed countries (Nainggolan, 2012; WHO, 2014). In Ghana, the increasing prevalence of hypertension and stroke, particularly in urban centres suggests that the burden of CVD's will continue to increase, placing a huge burden on the already overstretched health care resources.

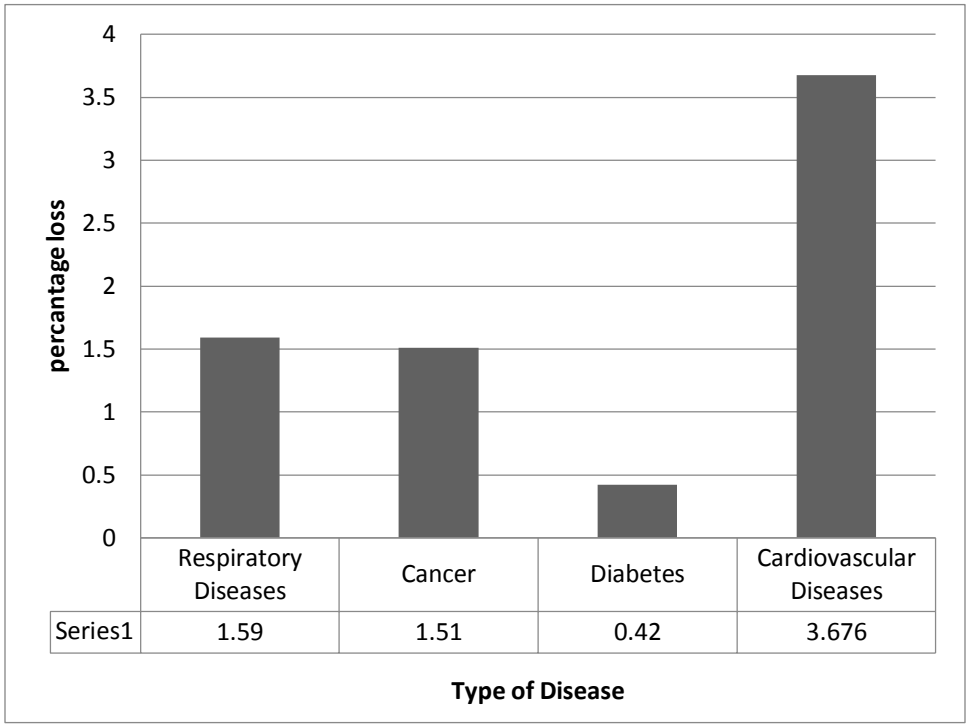

Figure: 2.1. Economic Loss of Non-communicable Disease in Low- and Middle-Income
(Source: World Economic Forum and World Health Organization, 2011)

2.5. PATHOPHYSIOLOGY OF CARDIOVASCULAR DISEASE

Age, high blood pressure (hypertension), high blood cholesterol, diabetes, overweight or obesity, lack of exercise, oxidative stress and family history have been associated with the occurrence of cardiovascular disease. The pathogenesis of CVD involves several mechanisms. The one resulting from obesity is complicated, however, according to De–Koning *et al.* (2007), the use of body mass index (BMI) as an indicator for obesity is debatable since it does not consider the distribution of fats in the body. He asserted that waist circumference was a better parameter comparatively to be used. He continued to indicate that a 1% increase in waist circumference will result in a 2% CVD risk and 5% CVD risk for waist to hip ratio. As to how obesity influenced dyslipidaemia, Vinik (2005) reported that, impaired adipocytes trap fatty acids to cause excessive adipocyte lipolysis. This in turn increases

the secretion of non-esterified fatty acids (NEFA) leading to an increase in hepatic lipogenesis. The results of this hepatic lipogenesis is an increase in enzymatic secretions, production of triglycerides (TG) and increased very low density lipoproteins (VLD)L ApoB circulatory levels necessary in atherogenesis (Bamba and Rader, 2007). It is interesting to note that generally HDL-C levels are reduced in obese individuals. What happens is that, in obese individuals, there is impaired lipoproteins lipase activity and an enhanced cholesteryl ester transfer proteins (CETP). These CETPs in exchange for TGs, transfer cholesteryl esters from HDL to triglyceride- rich lipoproteins (TRL) such that more cholesterol are siphoned from HDL to cause a lowered HDL-C level (Vinik, 2005; Bamba and Rader, 2007). Both authors concluded that increased TG, VLDL, LDL-C and a decreased HDL-C would result in 3 to 6 fold CVD prevalence.

According to Pashkow (2011), oxidative stress is identified as critical in the pathophysiology of atherosclerosis, thrombus formation and dyslipidaemia. In addition, elevated blood pressure and obesity have also been linked with oxidative stress (Wee *et al.,* 2008). In the case of oxidative stress caused by obesity, the increased body mass index (BMI) is known to initiate subclinical inflammation that causes an increase in reactive proteins, ultimately increasing the oxidative stress in vasculature (Wee *et al.,* 2008).

Similarly, the role that the gene (that regulates lifespan in mammals, reactive oxygen species (ROS) levels and induce apoptosis) p66SH and Protein Kinase C (PKC) play on oxidative stress in cardiovascular disease and obesity has again been established (De-Marchi *et al,* 2013). There is a correlation between obesity, CVD and oxidative stress and this is as a result of an imbalance between the production of reactive oxygen species (ROS) and cellular antioxidant defence system. In CVD, oxidative stress caused by lipid accumulation is pivotal in the pathogenesis of atherosclerosis (De-Marchi *et al*, 2013). Antioxidants, (enzymatic and non-enzymatic) are agent capable of inhibiting oxidation in oxidizable biomolecules. In addition, carotenoids and lycopene are also identified as

having the ability to decrease the initiation of oxidation. However some controversies have been raised in the use of vitamin E as an antioxidant; duration of use and high levels of the treatment to achieve results were some of the attributions. They finally concluded that, regardless of the controversial information from clinical studies, the prevention and therapy for oxidative stress remained a promising target with reference to CVDs. Inclusion of foods such as vegetables, fruits and certain foods known to contain phytonutrients could increase antioxidant capacity hence a reduction in CVDs.

2.5.1. Pathogenesis of Atherosclerosis

According to Antoniades *et al*. (2009), atherosclerosis development is orchestrated by the effect of non-esterified fatty acids (NEFA), pro inflammatory mediators and hyperinsulinemia that promote vascular wall oxidative stress, inflammation and dysfunction of endothelium. Reactive oxygen species (ROS) are generated as a result of lymphocytes and monocytes attaching themselves to the endothelial cells, causing the migration of leukocytes into the sub-endothelium (Greaves and Gordon, 2009). These cause an imbalance when the body is unable to detoxify them resulting in oxidative stress. The initiation of atherosclerosis occurs when oxidised low density lipoproteins are transported across the endothelium where damage caused by inflammation is, and into the arterial walls (Greaves and Gordon, 2009). Galkina and Ley (2009), added that, endothelial cells, smooth muscle cell and macrophages act as sources of oxidants in the modification of phospholipids in atherogenesis. These oxidised LDL then induces the adhesion of certain proteins to the damaged or injured site. As oxidised LDL take up macrophages, foam cell are formed. Combined with leucocytes and growth factors, the cells induce migration of smooth muscle cells into the lumen of arteries, calcifying with time to form plagues (Hahn and Schwartz, 2009).

2.6. ANTIOXIDANTS: A TREATMENT FOR CARDIOVASCULAR DISEASE

Malnutrition is becoming an issue of concern in Ghana and unhealthy diet a modifiable determinate of CVD, is one of the multifactorial risk factors for the disease (Lloyd-Jones *et al*., 2010; Micah and Nkum, 2012). Diets notably high in energy, low fibre, and high fats often contribute to cardio-metabolic determinants; dyslipidaemia, obesity, abnormal systolic blood pressure, hyperglycaemia and micro and macro vascular abnormalities (Appannah *et al*., 2015). In the World Health Survey 2002-2003, among the 52 low- and middle-income countries Ghana recorded the lowest fruits and vegetable consumption (Dake *et al*., 2011). Polished foods now replace unpolished ones, local foods almost abandoned and western diet now being in vogue; due to globalization were reasons Agyei-Mensah and De- Graft, (2010) observed.

Dietary antioxidants; as shown by studies have different chemical properties capable of recharging each other as in an antioxidant network hypothesis. Antioxidants are substances that reduce harmful chemical reactions promoted by oxygen. Despite the fact that, fruits, vegetables and their products have high total antioxidants properties they are less used in the Ghanaian communities (Agyei-Mensah and De- Graft, 2010). Fruits and vegetables that have a good source of vitamins C (ascorbic acid), E (tocopherol and tocotrienols) and dietary antioxidants need to be inculcated in dietary management for the treatment of CVD (Pashkow 2011; De- Marchi *et al*, 2013).

Many reviews have been carried out on antioxidants and its effect on lowering blood lipid (cholesterol) in dyslipidemic individuals. One of such reviews is by Mathers *et al.* (2016), they reported a reduction on total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and triglycerides after supplementing diet with vitamin C prominent anti-oxidant.

2.7. PHYTOCHEMICAL COMPOSITION OF *BORASSUS AETHIOPUM* FRUIT (PULP)

2.7.1. *Borassus Aethiopum* Fruit (Pulp)

The Palmyra palm *Borassus aethiopum,* simply known as Palmyra, is a tropical forest plant that bears about 50-100 fruits per tree. The fruit is greenish, turning dull orange-brown when ripe and approximately 8-18 × 6-16 cm in size and 1-1.5 kilograms in weight. The shape of the fruit, normally ovoid or triangular, is dependent on the number of seeds present, 2-3. The pericarp is tough, endocarp woody in nature with a yellowish pulp that is slightly oily, containing the seed. These seeds are 10 cm in diameter and 100 g in weight with a short viability (Small, 2011). The fruit has a pungent smell and bitter taste. It is eaten either raw or used as an ingredient in cookery (Ali *et al.*, 2010a).

2.7.2. Phytochemical In *Borassus Aethiopum* Fruit

The fruits contain several components**,** non-nutrients and nutrients alike. Adam **(**2005), simply describes nutrients as chemical substances in food that provide the human body with essentials to function. In addition, phytochemicals are bioactive, non-nutrient compounds found naturally in fruits, vegetables, and whole grains. They also contain pharmacologically active chemical compounds in various groups, polyphenols, organic sulphur containing and nitrogen compounds, carotenoids and alkaloids (Jeyaratnam, 2007; Varadarajen *et al*., 2008). Using aqueous and methanol extracts with various protocols, alkaloids, flavonoids, terpenoids, phenols, tannins, steroids and sterols, saponins, cardiac glycoside were found to be present in the pulp of the fruit (Amoateng *et al*., 2010; Poongodi Vijayakumar and Saranya., 2016 ; Ali *et al*., 2010b).

2.7.2.1. Effect of Cardiac Glycosides on CVDs

Cardiac glycosides belong to the steroidal family, are highly selective and have a strong affinity to other compounds. They are either produced endogenously in mammals or traditionally extracted from

plant compounds (Havis and Karlish, 2013). They act directly on the Na^+/K^+-ATPase pump by preventing energy from reaching it or interfere with its carrier mechanisms. Known to have a positive inotropic effect, it is used as an anti- arrhythmic agent on the heart muscles in cases of cardiac insufficiency. This effect, in the cardiac myocytes, act by inhibiting the sodium-potassium adenosine triphosphosphatase (Na^+/K^+-ATPase) pump, often mediated by an increase in Na^+ concentrations (Blaustein, *et al.,* 2006; Havis and Karlish, 2013). Furthermore, cardiac glycosides have been attributed to the Na^+ - lag hypothesis; which postulates that, a positive inotropic action on the cardiac muscles results in an increase in intracellular concentrations of sodium. This is implicated in the pathogenesis of heart failure and arterial hypertension. Poongodi Vijayakumar and Saranya (2016) and Amoateng *et al*. (2010), established the strong presence of these cardiac glycosides in *Borassus aethiopum* fruit extract when screened for phytochemical contents.

2.7.2.2. Effect of Saponins on Cardiovascular Diseases

Saponins are generally known as glucosides with foaming characteristics. A variety of plants contain certain natural surfactants in them. The surfactant activity of these plants is due to the presence of same molecule having both fat and water-soluble moieties (Yu *et al*, 2011). Studies have indicated that saponins have anti- atherogenic effect, attributed to its membrano-lytic properties; ability to biologically cause cell break down. The body uses cholesterol to produce bile necessary for digestion. However, Saponins also have the ability to bind and form micelles with bile salts and cholesterol influencing absorption of lipids (Cheeke, 2001). The process of reabsorption of cholesterol is impeded, it is thus excreted via the intestinal tract preventing dyslipidemia. Notably, many cholesterol lowering medications use this mechanism of action (Yu *et al*, 2011; Cheeke, 2001).

A stable foam is formed after vigorously shaking the extract with distilled water. The formation of an emulsion after 3 drops of olive oil has been added to the persistent stable foam is an indication for the presence of saponins.

2.7.2.3. Alkaloids and Cardiovascular Disease Treatment

Alkaloids have been studied over decades regarding their medicinal properties**.** They are nitrogen containing basic compounds with vasodilation effect and cholinesterase inhibiting properties (Andretic *et al.,* 2008). The vasodilation effect is endothelium- dependent and the vascular relaxing activity is felt directly on peripheral and cerebral blood flow contributing to hypertension hence cardiovascular disease (Laurienti *et al*., 2003).

Oxidation of alkaloids by sodium metaperiodate under mild acidic conditions forms an intermediate N, N'- dimethyl alloxan with MBTH (3-methyl 2- Benzo Thiazalinone Hydrazone Hydrochloride) which is measured spectrophometrically at 630 nm

2.7.2.4. Effect of Flavonoids and Tannins on Cardiovascular Disease

Several studies describe flavonoids as polyphenolic molecules, plant metabolites and phytonutrients that possess health benefits through cell signalling pathways. They can be in several form or groups, tannins inclusive. All groups have phenolic compounds responsible for the varied properties; antioxidants anti-inflammatory, anti-allergic, anticancer and anti-neoplastic for the treatment of several disorders (Okuda 1992; Hasty and Surmi 2008; Dalgard *et al.* 2009). Three mechanisms have been attributed to its anti-inflammatory and antioxidant activities. The presence of catechins and epicatechine in a diet is responsible for its anti-platelet effect; preventing the aggregation of platelets at site of plague rapture, in the pathogenesis of atherosclerosis (Pearson *et al.,* 2002). In addition, a reduction in lipid peroxidation is realised, mainly due to liquid soluble flavonoids interpolating the

membranes of lipoproteins (Wiswedel *et al*., 2004). Finally, Han *et al*., (1999), indicated that human lymphocytes are protected by the ability of the flavonoids to prevent oxidative damage in plasmin activity.

A charge- transfer resonance hybrid, stable in aqueous medium, interacts with sodium nitrate to form a pink colour that is measured spectrophotometrically at 510 nm. This hybrid is as a result of an attraction of atomic nuclei of the aromatic ring in the flavonoids and the positive nature of aluminum present in the extract formed.

Potassium ferricyanide reduced by phenols produces ferrous ions. The reaction of ferrous ions with ferric chloride in the presence of diluted HCl forms a Prussian blue coloured complex which is measured spectrophotometrically at 700 nm for the presence of tannins.

2.7.2.5. Effect of Sterols and Steroids on Cardiovascular Disease

Being an essential component of eukaryotic membranes, sterols play an important function in signal transduction, membrane fluidity and permeability. They are synthesised or taken from plant source. Plant sterols are steroid alcohols that have atherogenic properties. This is as a result of their ability to eliminate cholesterol by inhibiting its absorption (Helske *et al.* 2008; Vasanthi *et al*, 2012). In addition, sterols, have the ability to reduce concentrations of cholesterol in plasma (Schonfeld, 2010). The mechanism by which sterols inhibit the absorption of cholesterol was studied by Vasanthi *et al*, (2012). Vasanthi *et al*. (2012), noted that, esterified plant sterols are hydrolysed in the upper parts of the small intestine. These are then transferred into micellar phase as they journey through the first half meter of the intestine, half of the sterols are hydrolysed and the other half transferred into this micellar phase. Cholesterol in oil phase is increased as these micellar-phased sterols increases, resulting in retardation in cholesterol absorption, hence its elimination in stool.

In the presence of H_2SO_4, a phenanthrene anion is formed when acetic anhydride reacts with hydroxyl hydrgens of the sterols. A green coloured product measured spectrophotometrically at 640 nm is realized when the phenanthrene is combined with acetic acid.

2.8. NUTRIENTS IN *BORASSUS AETHIOPUM* PULP

Nutrients are building blocks of living organisms necessary for most body functions, growth and survival. They are either required in large (macro) or small (micro) quantities. Both nutrients are of vital importance to the development of cell structures and activity of the body. *Borassus aethiopum* fruit is noted for proteins (1.4%), fats (4.56%) and carbohydrates (72.93%) in its pulp and roots extract (Thimmaiah 1999; Vijayakumari, *et al.*, 2014; Adzinyo *et al.*, 2014). In addition, vitamin C, carotenoids and calcium are also present in the pulp of the fruit (Ali *et al*., 2010a,b).

2.9. USES OF *BORASSUS AETHIOPUM* PULP

The *Borassus aethiopum* plant is a multi-purpose plant. Almost all the parts of the tree are used for nutritional and non- nutritional purposes (Figure 2.2). Nutritionally, it is used as food eaten either raw or as an ingredient in cooking. Non- nutritionally, it is used ornamentally for baskets, fans and as

timber for carpentry (Ali *et al*., 2010a, b).

Figure 2.2. *Borassus aethiopum* eaten as a fruit in Ghana

2.9.1. Traditional

The Palmyra plant has several uses involving the roots, leaves and fruit (Gruca *et al*., 2015). The fruit of the plant are used as food either eaten raw or cooked for various reasons.

2.9.2. Pharmacologic

The plant has been identified to have stimulating, aphoristic, sedative, laxative, anti- plasmodia, anti-pyretic, anti-leprotic anti-inflammatory and antioxidant effects. In addition, it is used to treat fungal and viral infection especially measles, impetigo and asthma. Findings indicated that either the fruit, pulp from the fruit, leaves or roots were often used. The fruit is eaten raw or boiled, leaves and roots grounded and boiled or diffused in water with apparently no specified dosage (Siaw *et al*., 2014; Gruca *et al*., 2015).

2.9.3. Confectionery

A study revealed that the pulp could be used for beverages and processed into powder (flour) rendering it a good commodity in the bakery industry (Ali *et al*., 2010a, and b; Vijayakumari *et al*., 2014).

2.9.4. Technologic and physico-chemical

The pulp has good solubility index, rendering it highly viable for the production of flour for confectionery use (Ali *et al.,* 2010a and b). In addition to solubility, an increase in certain enzymes, temperature and time applied to pulp increases yield (Agbo N'zi,1992) Again, the pulp has good concentration properties when used to develop a ready to serve beverage (Nilugin and Mahendran, 2010).

2.10. ANTHROPOMETRIC AND BIOCHEMICAL MEASURES

2.10.1. Waist Circumference

Waist circumference (WC) is clinically used as a parameter in assessing abdominal adiposity (Ayala *et al*., 2014). This was confirmed by Janssen *et al*. (2004), when they noted that, abdominal obesity, a risk factor for CVD could be determined well with a powerful variable such as WC. The presence of abdominal obesity stimulate production of adipokines; involved in the regulation of appetite and satiety, energy expenditure and metabolism, activity, endothelial function, blood pressure and fat distribution, all linked to the developing cardiovascular diseases (Heiss and Goldberg, 2016). The cut-offs for abdominal obesity is classified as waist circumference above 102 cm for males and more than 88 cm for females (NCEP ATP III, 2002).

2.10.2. Visceral Fats

Visceral fats, also known as abdominal fats, belly fats or active fats are body fats stored deeper in the body. They are normally found around abdominal organs such as the liver and kidneys. Strongly linked with the progression of CVD's, visceral fats play a distinctive role in atherosclerosis, by secreting chemical that increase inflammation as well as regulate certain hormonal functions in the body (Mottillo *et al.,* 2010). In addition, visceral fats are directly associated to the increase in LDL-C and a reduction in HDL-C in the human vasculature (Alberti *et al.*, 2005).

2.10.3. Body Mass Index (BMI)

Body mass index, alternatively known as the Quetelet index was described as weight (kg) divided by height squared (m^2). It is usually used in the categorization of underweight, normal, overweight and obesity in adults (table 2.1). These values are same for male and female (NHMRC, 2013). Globally it is used to predict future health outcome of individuals (Ogden *et al.*, 2016). Age, sex and ethnicity of individuals, distribution of lean mass and body fat are limiting factors in its use (Zhu *et al*., 2014). A spectrum of chronic diseases including cardiovascular have been linked with obesity however BMI can help predict future health outcome and functional status of individuals (Grzegorzewska *et al.*, 2016).

BMI (Kg/m^2)

Categorization	**End point values**
Underweight	< 18.5
Normal range	18.5-24.9
Overweight	≥ 25.0
Pre-Obese	25.0-29.9
Obese	≥ 30.0
Obese Class I	30.0-39.9

Obese Class II	≥ 40.0

Source: Adapted from NHMRC (2013). .

2.11. ASSESSMENT OF LIPID PROFILE

Dyslipidemia is defined as single or a combination of irregularities in the blood lipid profile of individuals (Essah *et al*., 2008). This lipid disorders consist of high amounts of total cholesterol, triglycerides, low-density lipoprotein and a reduction in high-density lipoprotein (Niroumand *et al*., 2015). According to Ogden et *al.* (2006), the possibility of developing cardiovascular disease has been evaluated by assessing fasting blood lipid levels.

2.11.1. Total Cholesterol

According to National Cholesterol Education Panel ATP III (NCEP ATP III) (2002), cholesterol is the fat-like substance found in cell membranes. Cholesterol is transported into blood circulation via lipoproteins; very low, low and high cholesterols. High levels of cholesterol in blood is used as a marker for assessing atherogenic lipoprotein; low HDL and high LDL cholesterol. These atherogenic lipoproteins are variables for plaque formation in hypertension hence CVDs (NCEP ATP III, 2002). However high LDLs alters permeability of arterial endothelial which influence influx of LDL-C particles into arterial wall reducing cardiovascular risk. As such, high blood total cholesterol particularly VLDL and LDL can stimulate events of atherosclerosis (Pearson *et al*., 2002).

2.11.2. Triglyceride

Triglycerides are stored as bile emulsified fat in the small intestines. They are picked up in the intestines and carried by lipoproteins into hepatic tissue and adipose tissue (Austin *et al*,

1990). According to Rizzo et *al*., (2009) high triglycerides are used as marker of atherogenic dyslipidemia. Some studies have also reported that, hypertriglyceridemia are predictors of possible outcome of heart disease (Essah *et al.,* 2008). In addition, diets high in triglyceride have been proven to damage endothelial tissues rendering individuals at risk of initiating atherogenesis (Rizzo and Berneis 2005).

2.11.3. Lipoproteins Cholesterol

The lipoproteins found in serum of individual that have undergone fasting are classified as low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and very low density lipoprotein cholesterol (VLDL-C) (NCEP ATP III, 2002). They are protein transporter used to carry cholesterol in blood (Scanu, 1992). In human vasculature, LDL-C trigger oxidative process, increases the amount of reactive oxygen species that ultimately leads to damage of the endothelial cells and atherosclerotic events (Talayero and Sacks, 2011) predisposing an individual to the risk of cardiovascular diseases (Rizzo *et al*., 2009). Adversely, HDL-C protects the development of atherogenic plagues through its action. It reverses the transportation of cholesterol to liver for metabolism (Khan *et al.,* 2008). In most cases, a rise in concentration of HDL-C directly influences the risk of CVDs positively (Hwang *et al*., 2014).

2.12. SYSTEMATIC REVIEW

2.12.1. Literature Search

Borassus aethiopum is an indigenous tropical plant African found in the tropical zones, mainly around the eastern to western to southern parts of the continent. It grows almost anywhere, coastal plains, forest and drier grasslands but in abundance along banks of rivers or along water courses. It is a slow

growing and very long lived plant (over 100 years old) with almost all parts used. It provides food in a form of wines, oils and sap eaten either raw or cooked. Other uses include timber, fiber, dyes and leaves used as raw materials for mats and baskets. The plant has been identified to have stimulating, aphoristic, anti-leprotic, sedative, laxative, anti- plasmodia, anti-oxidant and anti-inflammatory effects (Siaw *et al*., 2014; Gruca *et al*., 2015).

A systematic search was performed from June to October 2016, from selected published papers on the Palmyra plant. The search looked at the African Palmyra plant, its parts, uses and constituents and / or properties. The electronic databases used in the search were PubMed, Google scholar and Plos one. In the respective databases, these articles were identified based on the search results: PubMed (4), Google scholar (345), and Plos one (4). The search words included antioxidant properties of Palmyra plant and risk of CVD, uses of Palmyra plant, Palmyra and CVD, phytochemical properties of *Borassus aethiopum*. After the 327 search articles have been thoroughly evaluated, most 73 were eliminated because they were only abstracts and citations, and 254 duplicating of articles, irrelevant information, partial data, and articles that did not meet inclusion criterion. A total of 8 articles, containing full text of publications were used and their contents evaluated (Figure 2.3). This was to evaluate the Palmyra plant with respect to its properties and uses.

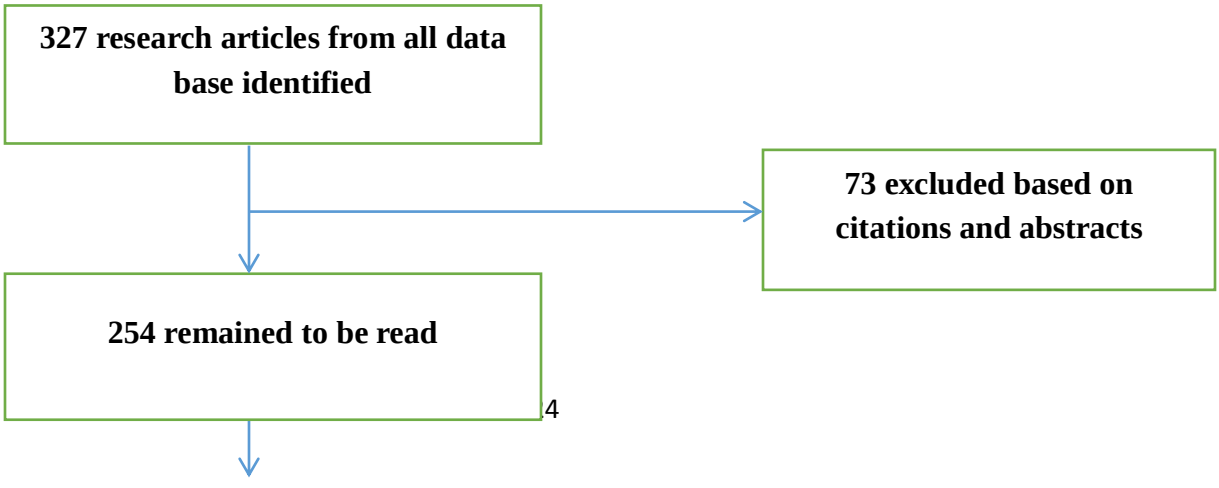

Figure: 2.3. Summarized search strategy

2.12.1. Inclusion Criteria

Study plan: descriptive, experimental, case-control, randomized clinical controlled trial

Study population: Human and plant (fruit) samples

Outcome: Outcome measured included parts of the plant, phytochemical, physiochemical and functional properties of the fruit as well as its uses.

2.12.3. Exclusion Criteria

Animal studies and studies on other parts of the plant aside the fruit.

2.12.4. Study Population and Country

Article reviewed had two different population; plant (fruit) and human beings. Studies that used humans (n=2) included both gender and an age range of 17-81 while other studies (n=6) used either the plant or its fruits. The predominant countries in the search results were Ivory Coast, Northern Cameroon, Burkina Faso, India and Ghana.

2.12.5. Study Design

Three study designs were identified in the articles reviewed. Four studies used descriptive design to find out the phytochemicals present in the fruit, possible uses of the fruit and the other parts of the plant. Three studies also looked at the strategies for preserving the fruit and its products and one a case

study to evaluate the anti-plasmodia effect of the plant using various parts including the pulp from the fruit.

2.12.6. Research Gap

From the result of the review, it was obvious that, much was not known about the plant hence, further studies need to be done in this regard. The phytochemicals present in the Palmyra had been researched into; its quantitative characterization was yet to be done. Even though the fruit had pharmacological properties, no randomised clinical trial had been conducted. Other useful properties of the plant particularly the fruit needs further study. Most methods of preservation had not been explored, with drying at varied temperatures the only method captured. Again, even though the fruit had been said to be edible, the toxicity level of the pulp (most frequently used) had not been looked at. Most of the products prepared from the fruit had no shelf life and microbial load determined with little bi-products explored. Though most of these researches have been done in Africa, not much has been done in Ghana especially with respect to using human population to determine the impact of the plant in treating some disease conditions. Summary of is in (Table 2.2).

2.12.7. RESULTS

Table 2.2 Summary of Author, Country, Title, Year, Aims and Sample studied.

Author and country	Year	Title	Aim	Study pop./ sample

Prabhakar PK Northern Cameroon	2015	Characterization of "soru-chakli"; a traditional food of west Benjal	Develop strategies for preserving and conserving traditional foods	20 fruits
Poongodi Vijayakumar India	2016	Preliminary phytochemical screening of raw and thermally processed palmyra palm (*Borassus aethiopum*)fruit pulp	Qualitative screening of phytochemicals	50 fruits
Ahmed Ali Northern Cameroon	2010	Physico– chemical properties of Palmyra palm (*Borassus aethiopum*) mart from North Cameroon	Evaluate the nutritional and morphological attributes and options for processing and storage.	100 fruits
Koffi E K Ivory coast	2010	Sensory analysis of the fruit juice of the Palmyra palm (*Borassus aethiopum);* A decision making tool	Assess the acceptability of product of *Borassus aethiopum* fruit	16
Agbo Nzi Ivory coast	1992	Characteristics of juice of palmyra palm (*Borassus aethiopum)* fruit	Develop an efficient process for the extraction of juice from the plant using pectinase	50 fruits
Saron Mathurin Burkina Faso	2007	*Borassus aethiopum* Mart	Description and uses of fruit	2 trees
Tiho Tagouelbe Ivory Coast	2017	Drying temperature effect on total phenols and flavonoids content and antioxidant activity of *Borassus aethiopum* mart ripe fruit pulp	Reduce perishability and enhance shelf life *Borassus aethiopum* fruit pulp	20 fruits
Marta Gruca Ghana	2015	Ethnomedicinal survey and in vitro anti-plasmodia activity of palm *Borassus aethiopum* mart	Activity of the component against the *P falciparium* (erythroctic stage)	50 human and In-vitro

Table 2.3. Summary of study design, main findings and knowledge gap

Study Design	Outcome measures	Main findings	Knowledge gap
Descriptive	Physical, surface and phytochemical characteristics of 'soru	The traditional food produced from the Palmyra can be preserved	Shelf life of the product was not evaluated

	chakli'		
Descriptive	Phytochemical compositions of raw and thermal processed Palmyra fruit pulp	Certain phytochemicals like glycosides, flavonoids, triterpenes, alkaloids and phenols were present in the fruit of the Palmyra plant Thermal treatment did not affect the phytochemicals found in the fruit Phytochemicals in the plant can be extracted and be used for pharmacological purposes.	Other methods preservation were not considered as well as determining their effect on the phytochemicals present
Experimental	Nutritional and morphological attributes of the Palmyra fruit pulp	The Palmyra plant had good nutritional values that made it a good source of vitamins C, pro vitamin A, sugars and some minerals. It can be used for other bi-products.	Further research to study the various perspectives attributed to the plant
Experimental	Acceptability of juice from pulp and determination of free sugars in fruit	The juice from the Palmyra plant had some bitter taste yet could be used as juice	Other parameters involving the use of other sense was not assessed
Experimental	How factors used in processing juice from the Palmyra affected yield and quality	Temperature used in treating, concentration of enzymes and maceration time affected the quality and yield of juice extracted from the Palmyra palm fruit	Other enzymes could be used in the process of extraction.
Descriptive	-	Major description of most of the parts of the plant and their individual uses	Other properties that the plant had were not explored. They did not find out other bi-products from the plant.
Experimental	Perishability of *Borassus aethiopum* fruit pulp	The *Borassus aethiopum* pulp could be preserved by drying maintaining its medicinal and nutritional properties	Various method of drying was not applied. Much research is recommended for further observation
Case study	Activity of component of Borassus on P. *falciparium*	*Borassus aethiopum* fruit had anti-plasmodia activity and could be used in alternative medicine.	Characterization of active ingredients in the fruit was not done, as well as testing for various drug resistant strains of the P. *falciparum.* Toxicity levels of active compound was neglected.

2.12.8. RESULTS AND DISCUSSION

The systematic review conducted included three descriptive, four experimental and one case study. The descriptive studies showed an in-depth description of the Palmyra palm plant. Saron and Sacande,

(2007) botanically described the plant as the tallest of palms with an edible and non-edible wide distributed use. The seed required no treatment for germination with a delayed reproduction as well as a reproduction period of twenty years. The plant however had a long viability. The phytochemical properties of the pulp were also seen in one of these studies indicating its good nutritional and non-nutritional properties. Several phytochemical were present in the pulp; these phytochemicals have several medicinal attributes such as antioxidants, anti-inflammatory, anti-allergic, anticancer, anti-neoplastic properties for the treatment of several disorders (Amoateng *et al.*, 2010; Gruca, 2015; Poongodi Vijayakumar and Saranya 2016; Tiho 2017). They added that, fruits and vegetables such as Borassus and their products were high in total antioxidants and have the potential of reducing diseases especially CVD caused by oxidative stress (Amoateng *et al.*, 2010; Tiho, 2017).

The experimental studies evaluated the possible products that could be obtained from the pulp and the effect of treatment on the nutrient and non- nutrient present. Ali *et al*. (2010), however identified only the making of juice and flour as product from the pulp.

The case study explored the effect of the pulp against the *P. falciparum.* A positive result was achieved indicative of the fact that the pulp had anti- plasmodia properties.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. STUDY DESIGN

A randomised single blinded-placebo controlled clinical trial method was conducted at the 37 Military Hospital in the Greater Accra region of Ghana. Participants were randomly placed into two groups, control and intervention. Participants were coded and codes placed in a draw with two people pulled from the draw alternatively with, each person representing a group. A placebo was given to the control group and this was bread prepared with same ingredients as used for the intervention with the exception of raw *Borassus aethiopum* powder (RBAP) replaced with food colouring to attain the same colour as that of the intervention. The size, shape, weight and packaging were same. The *Borassus aethiopum* composite bread was given to the intervention group, in order to evaluate the impact of the bread in lowering blood lipid and other parameters. The amount administered, the timings involved, and the quantity of *Borassus aethiopum* flour in the bread were monitored regularly. Participants were not aware of what was in the bread given at any given time. Constituents of both types of bread were known by only the researcher. Details presented in Table 3.1.

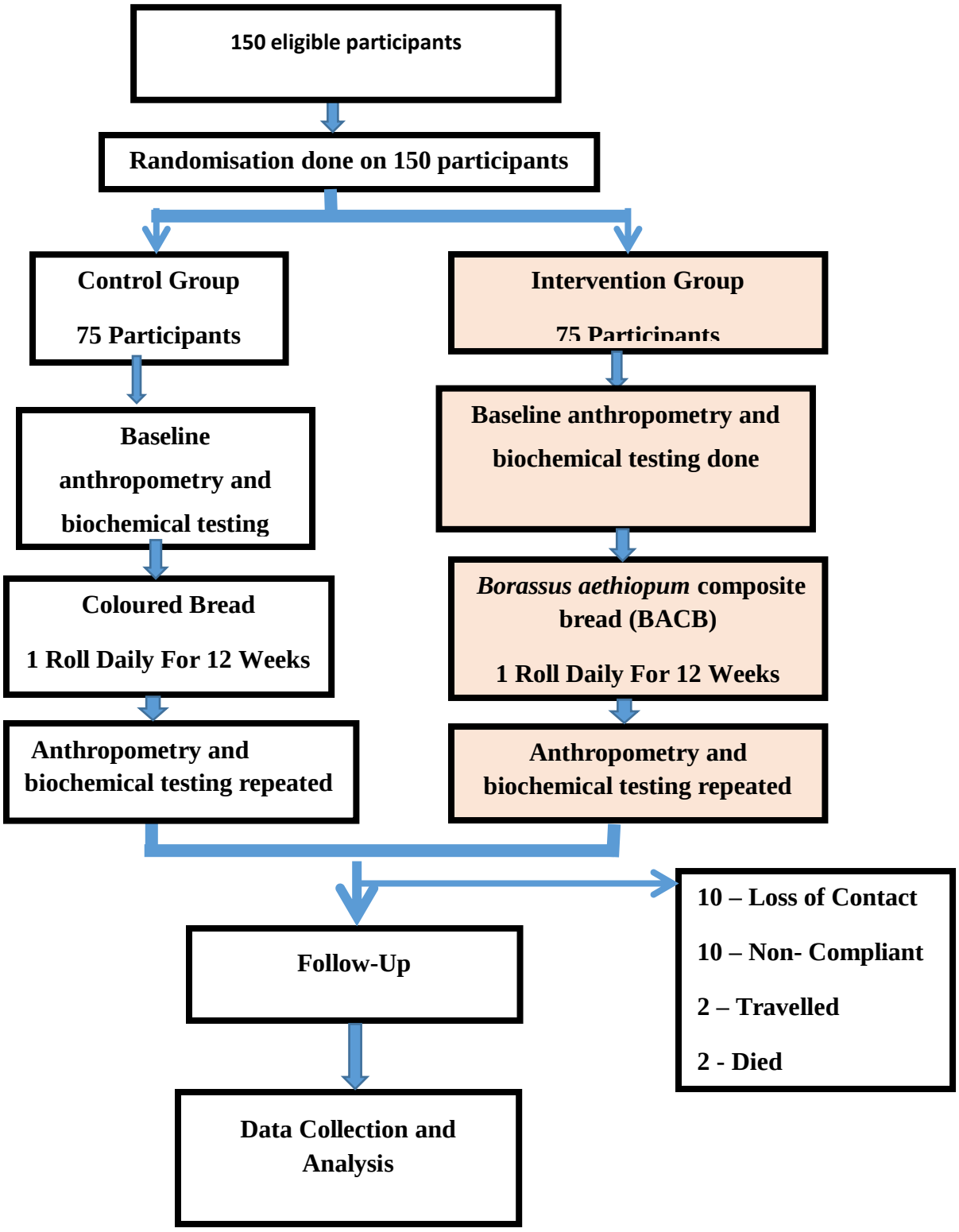

FIGURE 3.1: Flow chart of study design

3.1.1. Research setting

The project was carried out in three institutions, from May, 2016 to April 2017. With a tropical climate Ghana is located in the west coast of Africa, with about 70 different ethnic groups and a population that currently stands at about 27.4 million. (Ghana Demographics, 2017; WHO, 2014). Politically, the country is divided into 10 regions, with Accra in the Southern as its capital city. The WHO estimate for life expectancy for Ghanaians is, 61 years for male and 63.9 for female (WHO, 2014).

The research site was the 37 Military Hospital, one of the teaching hospitals in Ghana, Acumed Diagnostic Centre and Kwame Nkrumah University of Science and Technology's (KNUST) Food Science Laboratory located in the Greater Accra Region and located in the Ashanti Regions respectively. The hospital and medical laboratory are in the Ayawaso District in the Accra Metropolis respectfully.

Initially constructed in 1941 and re-designated in 1956, 37 Military Hospital had grown to become the second largest medical facility in Accra. It is a 600 bed capacity hospital with 13 wards (Ward 1 through to 10, F1, 2 and 3). Most of the services offered are on 24 hours, 7 days a week basis. Its main objective is to provide health care to service personnel and their families, civilian employees of the ministry of defence and their families, ex- service personnel as well as general public for a fee. Currently, it also serves as the government's emergency and disaster hospital and United Nations Level IV Hospital in the West Africa sub- region. The hospital served as the venue for recruiting participants (attendees of Medical Department), taking anthropometric information and blood samples and allocating bread (Diet Therapy Department).

3.1.2. Study Population

The study included only patients diagnosed of cardiovascular disease. A total of about 1000 patients report at the facility annually including newly diagnosed and old cases.

3.1.3. Sampling Technique

Participants were recruited by convenience from the Medical Outpatient Department (MOPD), polyclinics and wards of the 37 Military Teaching Hospital from November through to December, 2016. Participants who met the inclusion criteria (n = 150) were recruited. Questionnaires were taken to the hospital on appointed days agreed by the hospital administration. The questionnaires were given to the participants after a formal introduction, intentions made known and consent sought. Recruitment of patients was based on a definite clinical and or radiological diagnosis of cardiovascular related disease by medical personnel either on medications or not. On the other hand, patients with ambiguity of diagnosis, not medically diagnosed, not able to stand or walk without support, below 18 years and not attendees of the hospital were excluded from the study. In total, 150 eligible participants were recruited.

3.1.4. Sample size

Sample size of a study generally refers to the number of participants that are chosen from a targeted population (Lavrakes, 2008). Cochran's formula 1989 was used,

- $N= Z^2 p (1-p)/d^2$

N represents sample size

Z= confidence level = 95 % (Z-score standard value = 1.96)

p = Estimated cardiovascular (patients) prevalence = 10% = 0.1

d = marginal error

$N= 1.96^2 * 0.1 (1-0.1) /0.05^2$

N= 3.8416 * 0.1 (0.9) /0.0025

N= 138

A total of 138 participants should be used; however, in anticipation for dropouts, 150 participants were recruited for study.

3.2. PARTICIPANTS

3.2.1. Inclusion Criteria

Patients 18 years and above, attendants of the Medical Department of the 37 Military Hospital and medically diagnosed of having cardiovascular disease were included in the studies.

3.2.2. Exclusion Criteria

The study excluded attendants of the medical department of the 37 Military hospital who were medically diagnosed of having other conditions other than cardiovascular disease, relatives of patients, patients from other departments, patients less than 18 years and staff of the hospital were also excluded.

3.3. DATA COLLECTION TOOLS

Questionnaires (for sensory panel and participants) and a week's dietary recall form were used in this study**.** The first questionnaire was used for sensory analysis of the product (bread) to be used for the intervention**.** It had four sections, A, B, C and D to assess participants concept on olfaction, basic taste (sweet, sour, bitter, Salt and savoury), appearance and firmness respectively. Hedonic rating was deployed. The second questionnaire was in six (6) sections (section A, B, C, D, E and F). Section A was used to collect some basic information from the participants. Section B, C, D and E were used to collect information on current, past medical, family and social history respectively. Section F was however used for results of investigations, pre and post intervention and anthropometries. The questionnaires were pretested among ten patients from Korle - Bu Polyclinic to ensure that its feasibility to work with.

3.4. RECRUITING PARTICIPANTS.

Participants who met the inclusion criteria were recruited. Among the eligible participants, 70 were obtained through the Medical Outpatient Department of the hospital, 30 from the Polyclinic, 20 through the diet therapy unit and 30 from the various medical wards of the same hospital. Of the one hundred and fifty participants who enrolled in the study, ten were lost due to loss of contact (did not pick their phones when calls were made for promptings), another ten did not pick up the bread on scheduled day hence not compliant, two travelled to their hometowns for funeral and faced with financial constraints coming back and two were deceased in the course of the study. These were identified during the monthly blood pressure checks, weekly recordings of product (bread) allocations and final day for post intervention anthropometries and laboratory investigations. At the end of the entire five months; recruitment through intervention to post intervention anthropometries and laboratory investigations, 122 representing 81.33% of initial participant's fully participated in the intervention study with 18.6% dropout. Once a participant was recruited, a structured questionnaire was used to collect socio-demographic, past, family, social and medical history. Participants were given a scheduled date for baseline anthropometry and blood sample for biochemical analyses.

3.5. SAMPLE COLLECTION

Fully ripened Palmyra palm fruits (*Borrasus aethiopium*), with distinct flavours were randomly picked under some of the trees and others plucked with a cutter from Sekyi Odumase and St Mary's Sanctuary 'Obuohu" all IN the Ashanti region. The samples were transported to Kwame Nkrumah University of Science and Technology (KNUST) laboratory for further use.

3.6. PRODUCT DEVELOPMENT

3.6.1. Powdered Palmyra fruit

The ripe palmyra fruits were washed thoroughly, the outer thick skin (exocarp) peeled off and fibrous part with fruit pulp (mesocarp) separated from the seed. The fleshy layer was chopped into small pieces and spread in clean trays. The trays containing the fleshy fruit samples were placed in a convectional solar dryer for some days to dry. The dried samples were then milled into powder using a hammer mill with the various sizes of sieves and finally stored in black polythene bags at temperatures below 4^0C for further use.

3.6.2. Product (bread) development

Using Jamie Oliver's basic bread recipe with slight modifications in ingredient, quantities and methods, the raw *Borassus aethiopum* powder (RBAP) was used as composite flour for the preparation of the dough. Two different types of flour (white and whole wheat), were used in varied ratio with the RBAP. Other ingredients used for the product were, margarine, sugar, salt, instant yeast, diluted milk, nutmeg, egg and some drops of essences. To both types of flour, the ratio of flour to RBAP was 2:1, 5:1, 10:1 and 20:1 giving a total of 8 samples. The ingredients were carefully weighed, mixed to form dough, kneaded, dough weighed to give accurate weight per serving, allowed to double ferment for 4 - 8 hours, baked, cooled and finally packaged and stored till needed. Approximately 150 grams of dough was allowed per serving. Samples had varied characteristics after baking due to the varied ratios of flour to composite flour and types of flour used; this necessitated a sensory analysis to be done for the appropriate type of sample to be used for the study to facilitate compliance.

3.7. SENSORY ANALYSIS

According to Meilgaard *et al.* (1999), sensory analysis involves detecting a product, describing both qualitative and quantitative sensory components the product has by a panels of judges who could be trained or not. The aspect of a product that needs to be described involves the use of almost all senses. This includes aroma (olfaction), appearance (sight), flavour (taste), its texture (touch) as well as properties of a product that brings out its uniqueness allowing for optimization and validation. The type of analysis used was descriptive, with a total of twenty trained panelist drawn; five each from the 37 Military Hospital kitchen staff, dieticians, patients attending the diet therapy units and students from the Elizabeth Francis Sey Hall of the University of Ghana. Training was done a week prior to analysis on the frame of reference (illustration and defining the product attributes) and of comparison (standard language and type of ranking) as well as style of presentation. Analysis was done in two sections, 37 Military Hospital premises and University of Ghana campus. Questionnaire; the frame of comparison was handed to each panelist, after which coded sample were served them. The questionnaire had four sections, A, B, C and D. Section A dealt with olfaction with a hedonic ranking of pungent, minty, musky and astringent. Section B, was on basic taste (sour, salt, sweet, bitter and savory) with a ranking of like extremely, like very much, like moderately, neither like or dislike, dislike much, dislike very much and extremely dislike. Section C, appearance (sight) had a rating of very attractive, moderately attractive, not too attractive, and not attractive at all and finally section D, firmness (ability to hold) had the rating of very good, good, fair and poor. Samples were coded one through to eight for the bread variations.

3.8. ANALYSIS OF *BORASSUS AETHIOPIUM* FLOUR AND BREAD

3.8.1. Aqueous Extraction

The aqueous extraction was done by the protocol described by Shanmugum *et al.* (2010). Separately, 20 g each of sample (RBAP and PBAB) was added to 100 mL of water, boiled for 5mins and allowed to cool, filtered through Whatman no. 1 filter paper and volume adjusted to 100 mL. The extract was then used for the various qualitative tests.

3.8.2. Methanol Extraction

The alcoholic extraction method according to Cowan (1999), was used for methanol extraction of RBAP and PBAB separately. The bread was dried and milled; 20 g each of samples were soaked in 200 mL of methanol for about 24 hours. The slurry was then filtered through Whatman no. 1 filter paper and washed with 50 mL of methanol. The extracts were used for the qualitative screening of phytochemicals.

3.8.3. Phytochemical screening of *Borassus aethiopum* flour and bread

The modified Prussian blue, Salkowski, libermann Burchard, Shinoda, Dragendoff's and froth test Shanmugam *et al.* (2010), were used in the phytochemcial screening of both aqueous and methanol extraction of RBAF and BACB.

3.8.3.1. Test for Alkaloids (Dragendorff's test)

One milliliter of the Dragendorff's reagent was added to about One milliliter of the crude extract and mixed gently. Appearance of a dark orange or orange – red precipitates was indicative of the presence of alkaloids. For the Dragendorff's reagent, the following method was used;

Solution A: 1.7 g basic bismuth nitrite in 100 mL water or acetic acid (4:1)

Solution B: 40 g potassium iodine in 100mls of water

Mix 5 mL solution A, 5mls B, 20 mL acetic acid and 70 mls water

3.8.3.2. Test for Flavonoids (Shinoda test)

Eight to ten drops of concentrated HCl and a pinch of magnesium powder were added to one milliliter crude extract. After boiling for about 10-15 minutes and cooled, a red coloration was observed in the extract. This was an indication of the presence of flavonoids. This coloration normally disappears on standing.

3.8.3.3. Test for Terpenoids (Salkowski test)

Three milliliters of concentrated sulphuric acid was carefully added to a mixture of five milliliters extract and two milliliters chloroform. A yellow colour ring was formed at the interface of the two liquids. After about two minutes, this turns reddish brown, indicating the presence of terpenoids.

3.8.3.4. Test for Cardiac glycosides (Keller-Killani test)

To 1 mL crude extract, 1 mL of 1% SO_4 and 0.5ml 20% NaOH was added. Fehling's solution was gradually introduced and the mixture heated in a hot bath for 5 mins. A brick red coloured ring at the interphase indicated the presence of cardiac glycosides (Ndam *et al.*, 2014)

3.8.3.5. Test for Saponins (Froths test)

To ten milliliters of crude extract, five milliliters of distilled water was added in a test tube and vigorously shaken. A stable foam was formed after leaving it to stand for about three minutes. An

emulsion was formed after 3 drops of olive oil was added to the persistent stable foam which was an indication of the presence of saponins.

3.8.3.6. Test for Phenols (lead acetate test)

One milliliter crude aqueous extract was mixed with one milliliter of 0,008 M solution of lead acetate. White precipitate indicated the presence of phenols.

3.8.3.7. Test for Tannins (modified Prussian blue test)

One milliliter crude aqueous or methanol extract was mixed with one milliliter of 0.008 M potassium ferricyanide and 1ml of 0.03 M $FeCl_3$ in M HCl. A blue coloration indicated the presence of tannins.

3.8.3.8. Test of Steroids and sterols (Libermann Burchard test)

Half a milliliter of the extract was dissolved in two milliliters of chloroform and equal volume of concentrated sulphuric acid and acetic anhydride was added gradually along the sides of the tube. The turnings of red in the upper layer and yellow in the lower with green fluorescence indicated the presence of steroids and sterols in the extract.

3.8.4. Total phenolic content determination

Stock solution of the extract was prepared by dissolving 10 mg each of the dried samples in 1 mL water. 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 9 mL 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-Ciocalteu 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.

3.8.5. *In vitro* determination of antioxidant content in Borassus powder and bread

Stock solution of the extract was prepared by pouring 10 milligrams of the dried bread in one milliliter of water. Also, stock solutions of 10 mM of standard (Ascorbic acid) and 0.5 mM of DPPH was prepared by dissolving 0.176 milligrams of Ascorbic acid and 3 milligrams of DPPH in one milliliter of water and fifteen millilitres of absolute methanol, respectively. Complete dissolutions were achieved after solutions were vortexed. 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/L.

Hundred microliters of each concentration of the test sample was transferred into a 96 well plate. This was followed by the addition of 100 μL 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 mM in distilled water. Blanks (Distilled water) were used and experiments were performed in triplicates. Aluminium foil was used to cover the plates, shaken gently and plates stored in a dark place for 20 minutes. The absorbance was later read on a Synergy H1 plate reader at 517 nm. The scavenging activity in percentage was calculated using the following formula;

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

Nonlinear regression analysis was used to determine the effective amount of antioxidant (concentration at 50% (EC_{50}) necessary to decrease 50% of the initial DPPH concentration by.

3.9. MICROBIAL LOAD OF RBAP and PBAB

3.9.1. Microbiological assay

The microbiological assay was carried out at the Microbiology Laboratory of the Department Of Biochemistry And Biotechnology, KNUST. The flour was sealed in a plastic container and transported to the laboratory at ambient temperature for analysis. The microbial tests were Total Aerobic Count (TAC), Total Coliform count (TCC), yeast and moulds count, *S. aureus, E.coli* and Enterobacteriaceae.

3.9.1.1. Chemical Reagents

The agars used were products of OXOID Laboratories, Basingstoke Hampshire and England. They included Plate Count Agar used for the enumeration of total viable count; Brilliant *E.coli*^{TM} agar for *Escherichia coli*; Mannitol Salt Agar for isolation of *Staphylococcus*, Violet red bile lactose agar

(VRBLA) for the enumeration of total Coliforms and Violet red bile glucose agar for the enumeration of Enterobacteriacea.

3.9.1.2. Preparation of Plate Count Agar

Plate Count Agar (Nutrient agar) was prepared by suspending 23.5 grams in 1000 ml (1 liter) distilled water and heated to boil to dissolve completely. The media was then sterilized at 121^0 C for 15 minutes in an agar bottle and left to cool at 50^0 C before pouring into sterile Petri plates.

3.9.1.3. Preparation *Escherichia coli* medium

The presence of *E. coli* in the samples was confirmed and enumerated on the Brilliant E. coli/Coliform Selective medium. The agar was prepared according to the directive of the manufacturer; by adding 28.1g in a liter of distilled water and brought to boil (no further sterilization needed). The agar was cooled at 50^0C and poured into sterile agar plates after which sterility check was conducted for 24 hours.

3.9.1.4. Preparation of Selective media

The selective agars used were employed in the isolation and enumeration of Coliforms and Enterobacteriaceae using Violet red bile lactose agar and Violet red bile glucose agar respectively. These were prepared by dissolving a gram of the agar powder in distilled water and brought to the boil for 20 minutes at 100^0 C. The agar was then cooled to 50^0 C and poured into sterile Petri dishes.

3.9.1.5. Sample Preparation

A pre-weighed mass of ten grams (10 g) each of the flour and bread sample were weighed seperately and aseptically taken into a sterile jar containing 90 ml sterile bacteriological peptone as diluent. The

resulting solution was mixed and subsequent dilutions were prepared to obtain a six fold dilution for the microbiological analysis.

3.9.1.8. Microbiological Analysis

The various agars prepared for the different assays were inoculated with 100 µL aliquot of the samples solutions in triplicate using the spread plate technique with the exception of the fungi analysis where the inoculum volume was increased to 1000 µL using a pour plate method. The inoculated agar plates were incubated at 37^0 C for 24 hours and observed for the presence of visible colonies for the bacterial analysis.

The fungi plates were incubated at 25 ^{o} C for 72 hours for the yeast analysis and 120 hours for the mould assay.

3.10. ANTHROPOMETRIC MEASURES

3.10.1. Introduction

All measurements for the study were obtained at baseline and at 3 month time, however, blood pressure was monitored monthly for the three month duration.

3.10.2. Blood pressure

Blood pressure readings were obtained by investigator using an automated adult device with manually inflated mode sphygmomanometer (ADC 703 diagnostix palm aneroid) and a stethoscope (ADC platinum cardiology) was used. Required standard protocol (Table 3.1), was used for the classification of systolic and diastolic measure obtained.

TABLE: 3.1 Reference ranges for systolic and diastolic blood pressures

CATEGORY	SYSTOLIC, mmHg	DIASTOLIC, mmHg

Hypotension	Less than 90	Less than 60
Desired	90–119	60–79
	90–129	60–84
Prehypertension (high normal)	120–139	80–89
	130–139	85–89
Hypertension stage 1	140–159	90–99
Hypertension stage 11	160–179	100–109
Hypertensive urgency	≥ 180	≥ 110
Isolated systolic hypertension	≥ 160	< 90

SOURCE: The American Heart Association, 2017

3.10.3. Height

A manual, wall-mounted stadiometer (Seca 213 mobile stadiometer, Germany) was used in taking height. Participants were asked to take off their shoes and mount on the device with their back to the wall looking directly forward. It was ensured that, their heel, buttocks, upper back and occiput touched the stadiometer and directly underneath the drop down measuring device. The drop device was then lowered, resting gently on the participants head and measurement recorded in meters.

3.10.4. Waist circumference

The waist circumference was taken by the use of a simple measuring tape. The tape was placed around the abdomen, between the upper hip and lowest rib, ensuring that it does not snug or compress the skin. Measurement was recorded in centimetres; however 1 cm was deducted from measurement with respect to clothing worn.

3.10.4. BMI, Body fat, Visceral fat, Metabolic age and Muscle mass

These were done by the use of the full body sensor (Omron). It was used to obtain participants weight, body mass index (BMI), percentage body fat, percentage muscle mass, visceral fat and metabolic age. After the actual age, gender and height of participant was fed into the device. Table 3.2 was used to classify figures obtained for visceral fat.

Table: 3.2 Visceral Fat classifications

Visceral Fat Levels	Visceral Fat Classifications
9	0 (Normal)
10 – 14	+ (High)
≥ 15	++ (Very high)

3.11. BIOCHEMICAL ASSESSMENT

3.11.1. Lipid profile

After 8-12 hour fasting, blood test was also done same day, for baseline and post interventional recordings. Fasting lipid panel (total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides (TG)) were determined. For uniformity of equipment, a central laboratory was used for testing all blood samples taken. Blood samples were taken

from one of the consulting rooms of the Diet Therapy Unit. Five millilitres of arterial blood was drawn from each participant and placed in gel separator test tube by phlebotomist of the 37 Military Hospitals. Blood sample were transported to the Central Laboratory of the hospital in tube holders and centrifuged to separate the blood serum or plasma from the formed elements. Two millilitres of serum was pipetted into a cyro tube and placed in a cold compartment (freezer) below 0^0C. At the end of the four days, blood serum was transported in an ice chest with ice cubes on it to the Acumed Diagnostic Centre for lipid panel testing.

3.11.1. Profiling of lipids

The fully automated ACE Alera Clinical Chemistry Analyzer and its reagent, manufactured by Alfa Wasser-mann Inc, (USA) was used for the test. The reagents are stored on board the analyzer. After blood samples were loaded in trays, a pipette automatically aspirates the exact measured aliquot and discharges it into a vessel. Reagents were added from an on-board refrigerated supply, mixed, incubated and then passed through a colorimeter for absorbance to be measured. The concentration of the analytes is then measured. A bar- code scanner will then read the test after operator programs the required test. Results were automatically displayed on the screen of a monitor attached to the analyzer. Classification of results was done using the NCEP ATP 111 guidelines (Table 3.5). The test and anthropometric results were used to assess participants' possible changes in values in response to the intervention given.

Table: 3.5 Classification of Lipid profiles by NCEP ATP III, (2002)

Lipid Parameter	**Defining levels in mmol/L**
Total Cholesterol	< 5.18 Normal 5.18-6.16 High optimal ≥ 6.20 High
Triglyceride	< 1.69 Normal 1.69-2.24 high optimal 2.25-5.64 Abnormal

	≥ 5.65 Very abnormal
HDL-Cholesterol	< 1.03 Abnormal
	≥ 1.03 Normal
	< 1.3 Abnormal
	≥ 1.3 Normal
LDL-Cholesterol	< 2.59 Normal
	2.59-3.34 Near normal
	3.37-4.12 High optimal
	4.14-4.89 Abnormal
	≥ 4.92 Very Abnormal

3.11.1.1. Total Cholesterol

Determination of TC is achieved at 500 nm absorbance through the hydrolysis of Cholesterol ester and oxidation of 3 hydroxyl group. The chromophore for absorbance is achieved using H_2O_2 in a peroxidase catalyzed reaction. The cholesterol concentration is proportional to the Absorbance or colour intensity.

3.11.1.2. Triglycerides

TG concentrations are measured at 500nm using the same chromophore used in TC absorbance.

A cascade of reactions are used to generate the chromophore with the key reaction being the generation of peroxide in situ. Triglycerides are hydrolyze into glycerol and fatty acids using lipase. The carbon trio is then phosphorylated and oxidize to dihydroxyacetone phosphate with the release of H_2O_2.

3.11.1.3. High density lipoprotein (HDL) cholesterol

HDL is measured directly by rendering it inert to enzyme responsible for Cholesterol hydrolysis and oxidation under the assay conditions. The apoB which contains the lipoproteins are reacted with a blocking agent called sulfated α- cyclodextrin in the presence of Magnesium. The HDL complex is hydrolyzed and oxidized to produce Cholestenone and H_2O_2. The H_2O_2 is used to generate the chromophore qunoneimine which has absorbance at 600 nm.

3.11.1.4. LDL-Cholesterol

LDL-cholesterol was calculated from measured values of total cholesterol (total chol), triglycerides (TG) and HDL-cholesterol (HDL-chol) according to the relationship:

[LDL-chol] = [total chol] - [HDL-chol] - [TG] /5

3.12. INTERVENTION

3.12.1. Baseline Anthropometric and Biochemical Assessments

The intervention started on the Thursday 5th January, 2017 through to Monday, 10th April, 2017, a three month period. On scheduled days, participant's blood pressure, weight, height, body fats, visceral fats and BMI were taken. For proximity and convenience, two phlebotomist from the 37 Military Hospital were engaged in the blood sample taking. Two personnel in the Department of Diet Therapy Department, trained for the previous two months, took the anthropometries. All eligible participants prior (week) to start of intervention were put into two main groups. The process of grouping was done by putting all the codes that represent the participants in a draw. Two people were made to pull from the container with the coded paper. One person represented the control group and the other interventional group. Pulling of the papers was done alternatively till all 150 coded papers were drawn. The codes were recorded 75 each for intervention and control. On the 1st - 3rd of January, all participants in the interventional group were given a phone call reminding them of the need to attend the hospital for start of the study. The group was divided into two, half the number to come on Thursday and the other Friday to prevent congestion. This was strictly according to participant choice when the call was made. They were also informed of what was to be done that day, anthropometries, laboratory testing (lipid profiled) and bread collection. Information was also given on the need to fast for a period of 8-12 hours (last meal by 8pm, 4th and 5th January) before the test day. The same

procedure was repeated for the control group on the Monday and Tuesday, 5th and 6th (last meal by 8pm 8th and 9th January) of the same month.

3.12.1. Allocation of bread

On Wednesday, the 4th of January, the first interventional bread (PBAB) was prepared and was to be prepared on every Wednesdays and be collected on Thursdays. Baking was carefully done, with regards to correct weighing of ingredient, dough measurement and packaging. Similarly, the control group had their bread prepared on Saturdays to be collected on Mondays. A blinded placebo was given them. This was also bread without *Borassus aethiopum* flour, prepared from white flour and food colouring at a dough weight of 150 grams approximately. All packs contained 8 rolls each. Each participant was to be given one pack each to be eaten a roll a day at night conservatively for the stipulated 90 days. Collection of bread was done at the Diet Therapy Unit of 37 Military Hospital on Thursdays (intervention group) and Mondays (control group). Participants were required to sign against their names before collection of bread. This was necessary to be used as checks on participant's compliance. Monthly phone calls were also made to participants to discuss progress, address any challenges and provide some encouragement.

3.13. STATISTICAL ANALYSIS

Data was entered on Microsoft office 2010 excel sheet and transferred to SPSS (Statistical Package for the Social Sciences) version 23 for analysis. Results were presented on chats, tables or graphs. Descriptive analysis stratified by group and gender was performed to assess frequencies of demographic characteristics, anthropometries and biochemical data of study subjects. Cut-off points were used for prevalence of some anthropometric and biochemical parameters. Paired T- test was used to compare means ± standard deviation, differences between the test groups and control, baseline and

after three months intervention. Comparism of means within groups was also done using unpaired t test, chi square or fisher's exact test where appropriate. Partial and bivariate correlation was used to find association between lipid parameters, and anthropometry data. Correlation was significant at 0.01 and 0.05 (2- tailed). Analysis of variance (ANOVA) was used to determine the effect of medication on anthropometries and lipid parameters. All analyses were significant at p value $\leq$ 0.05 and 95% confident level.

3.1.4. Ethical consideration

The 37 Military Hospital Institutional Review Board and Committee on Human Research, Publication and Ethics, approved the protocols, and gave an ethical clearances for commencement of study. The purpose of study was made known to all participants. Assurance was also given to the participants of confidentiality of information given and finally their consent sought by the signing of a consent form. There was no discrimination whatsoever among participants and any information got was not identifiable. During the days of ansalysis, all identifiable information was stored in a security code laptop only accessible to researcher.

CHAPTER FOUR

4.0. RESULTS

4.1. PHYTOCHEMICAL AND ANTI-OXIDANTS SCREENING

4.1.1. Phytochemicals Screening

The aqueous and ethanol extracts of RBAP and PBAP were screened for possible phytochemicals including terpenes, cardiac glycosides, saponins, alkaloids, flavonoids, phenolic, tannin, steroids and sterols. The results of both aqueous, ethyl and ethanol extraction are presented in Table 4.1. Terpenes, cardiac glycosides, saponins, alkaloids, flavonoids, phenolic, steroids and sterols were present in either ethyl, ethanol or aqueous extracts of the raw *Borassus aethiopum* powder and *Borassus aethiopum* composite bread (BACB), however, tannins were absent in all extract and samples used.

Table: 4.1. Phyto-constituents of samples

	RBAP			BACB	
Parameters	**Ethyl extract**	**Ethanol extract**	**Aqueous extract**	**Ethanol extract**	**Aqueous extract**
Flavonoids	Present	Present	Present	Absent	Present
Saponins	Absent	Present	Present	Present	Present
Glycosides	Absent	Present	Present	Present	Present
Alkaloids	Absent	Absent	Present	Present	Present
Tannins	Absent	Absent	Absent	Absent	Absent
Steroids And Sterols	Absent	Absent	Present	Absent	Present
Triterpenes	Absent	Present	Present	Present	Present
Phenols	Present	Present	Present	Present	Present

RBAP: raw *Borassus aethiopum* powder BACB: *Borassus aethiopum* composite bread

4.1.2. Total Phenols in *Borassus aethiopum* Powder and *Borassus aethiopum* Composite Bread

The determination of the level of total phenolic was not based on the absolute measurements of the amount of phenolic compounds, but on their chemical reducing capacity relative to gallic acid. The

results of total phenolic found in the *Borassus aethiopum* composite bread was comparatively lower than the quantity in the *Borassus* powder, as presented in Table 4.3. In both samples, the amount present was less than the standard gallic used, and significantly different (p= 0.007 and 0.000) in relation to the standard.

Table: 4.2. Total Phenols in *Borassus aethiopum* powder and bread

Sample	Total Phenolic (mg/mL)	P value
Gallic acid	4.9 ± 0.02	
RBAP	1.1 ± 0.55	0.007
PBAB	0.7 ± 0.11	0.000

RBAP: raw *Borassus aethiopum* powder PBAB: powdered *Borassus aethiopum* bread

4.1.3. Anti-oxidants activity of *Borassus aethiopum* Powder (RBAP) and *Borassus aethiopum* composite Bread (BACB)

The free radical scavenging activity of both the flour and the composite bread samples were tested through the DDPH method and the results are presented in Table 4.3. The role of antioxidants and their interaction depend on oxidative free radicals. In the present studies, the flour and the bread samples were able to decolourise DDPH. The free radical scavenging potentials of the samples were found to be 2.11±0.02 for RBAP and 2.24±0.41 for PBAB. In both samples, the EC_{50} activity in the RBAP and PBAB was significantly (p=0.00) though lesser activity than that of the standard ascorbic acid 0.12 ± 0.02 when compared with the RBAP having a higher EC_{50} activity compared to the BACB.

Table 4.3. Antioxidant properties of raw *Borassus aethiopum* powder and bread

Sample	Mean EC_{50}	P value
Ascorbic Acid	0.124407 ± 0.022204	0.000
RBAP	2.111457 ± 0.242506	
PBAB	2.242561 ± 0.416336	0.000

RBAP: raw *Borassus aethiopum* powder BACB: *Borassus aethiopum* composite bread

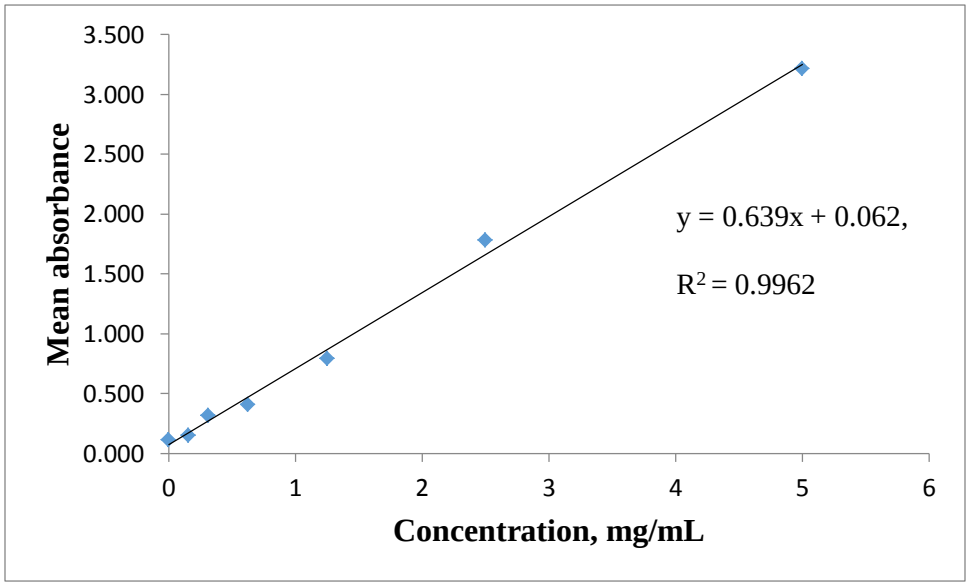

Figure 4.1: Standard concentration curve of absorbance against concentration of Standard Gallic Acid

4.2. MICROBIAL PROFILE OF FLOUR AND COMPOSTE-BREAD

4.2.1. Bacteria Assay

The results indicated the presence of aerobic microorganisms in the sample for the total aerobic count assay. The detected counts were, however, within the safe or acceptable limit of 1.0×10^4 cfu/g as prescribed the International Organisation for Standards, ISO (Centre for Food Safety, 2014). The detected aerobic count was averagely 1.75×10^3 ±7.07 cfu/g for the flour and 2.31×10^3 ±7.07 cfu/g for

the composite bread sample. The biochemical profile of the isolated colonies from the aerobic plates indicated the isolates to be predominantly *Bacillus* spp. but not *Bacillus cereus* from the biochemical profile (Plate 4.1).

No food pathogens (*E. coli, S. aureus,* Enterobacteriaceae and Coliforms) was detected in the flour sample. The biochemical profile of the isolated colonies from the aerobic plate indicated the isolates to be bacteria and of the *Bacillus* species and some Gram negative rods.

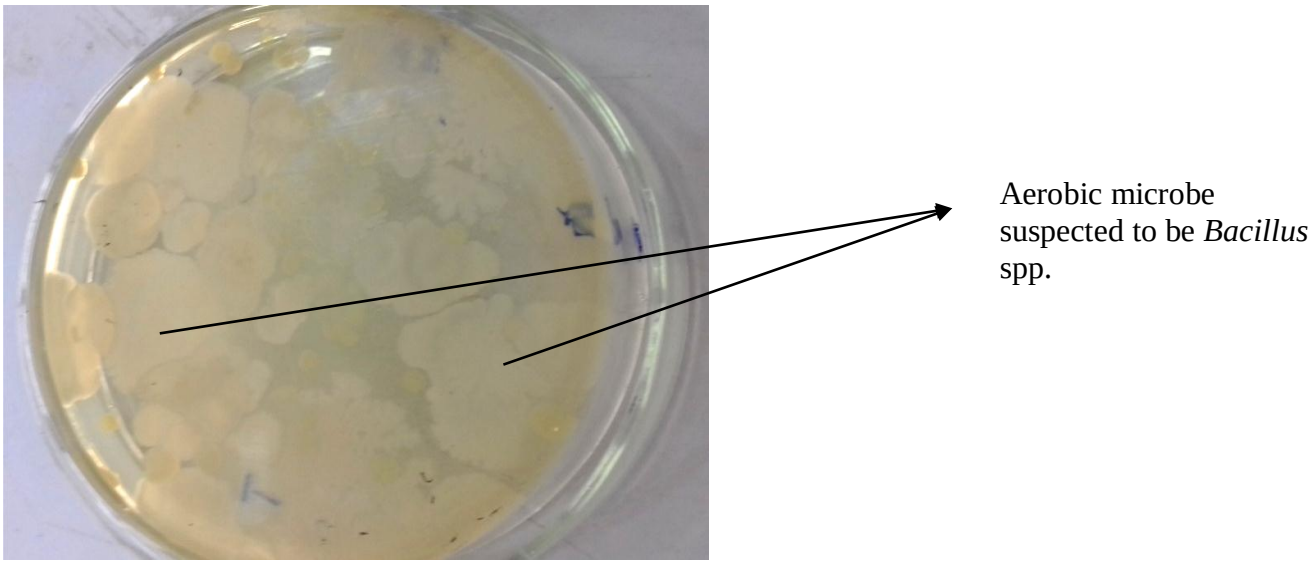

Plate 4.1: A representative plate showing aerobic microbe on PCA

4.2.2. Fungi assay

No mould was detected in the flour sample, however, one was found in the composite bread sample after analysis. The yeast cells detected were <10 and thus considered too few for cfu determination.

4.3. SENSORY ANALYSIS

The interpretation of data collected was done from results displayed from SPSS. A total of 20 panellists were recruited. Eight samples were given to each panellist for each section or sub-section. A total of 160 samples was analysed for each parameter. The interpretation of the data was done with respect to the preferred type of sample and parameter.

From the outcome of the sensory analysis done, sample three and four were the most preferred sample, however sample 3 was used with the reason that double of sample 4 had to be consumed in order to get the quantity of RBAP in sample 3. Sample three constituted white flour and composite RBAP in the ratio of 10: 1 at a dough weight of approximately 150 grams. This estimate provides the participant with at least 5.6 grams of RBAP per bread roll.

4.3.1. Olfaction (smell of the composite bread)

From the total sample, 113 representing 70.6% indicated that, the sample had a pungent smell, (38) 23.75% musky and (9) 5.62% astringent. Out of the 113 response, the preferred samples were 3 and 4 (20 each). In conclusion, panellist suggested that the *Borassus* bread had a pungent smell but the samples 3 and 4 were preferred (Figure 4.2).

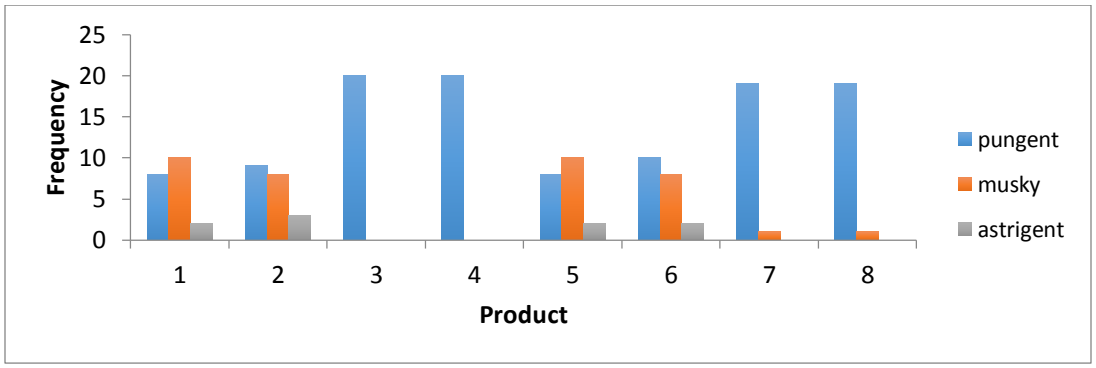

Fig 4.2. Frequencies of choice made for the scent of the composite bread (Olfaction)

4.3.2. Basic taste of the composite bread

With regards to basic taste, (section B), the parameters used were sour, salt, sweet and bitter.

4.3.2.1. Sourness of the composite bread

Total response for samples for sour (160) was 159, representing 33.12%, 53 moderately liked the sour taste. Among these, the preferred samples were 3 and 4 (Fig.4.3).

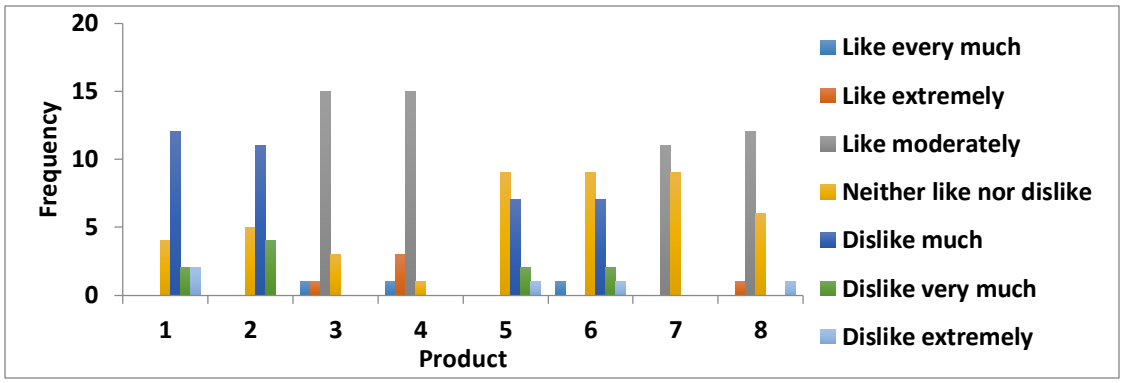

Figure: 4.3. Frequencies of choice made for Sourness of the composite bread

4.3.2.2. Saltiness of the composite bread

With a total sample of 160, (81) 50.62 % moderately liked the salt content of the bread. The preferred samples were o (12) 24.69% from sample 7, and (11) 13.58% sample 3 (Fig.4.4).

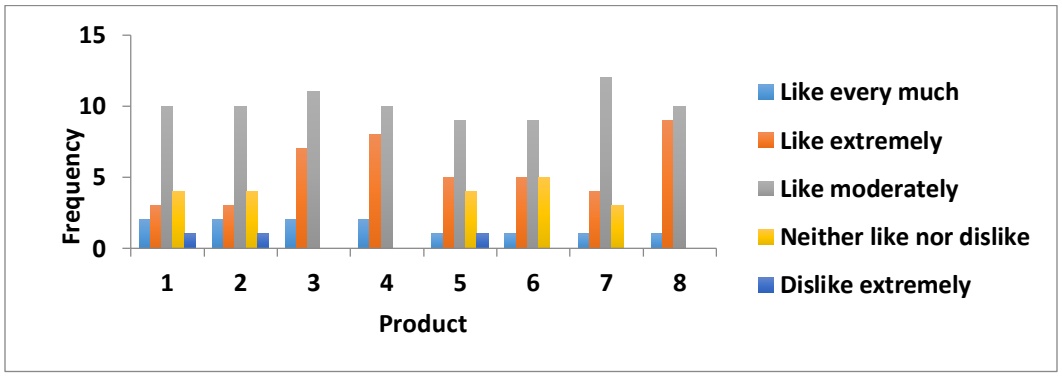

Figure: 4.4. Frequencies of choice made for the Saltiness of the composite bread

4.3.2.3. Sweetness of the composite bread

Five parameters were used to determine the sweetness of the product; extremely like it, like very much, moderately like, neither liked nor dislike, dislike and extremely dislike the sample. Of the 160 samples, 60 representing 37.5% moderately liked the sweet taste of the bread. Ten (16. 66%) were from samples 5 and 6 (Fig.4.5).

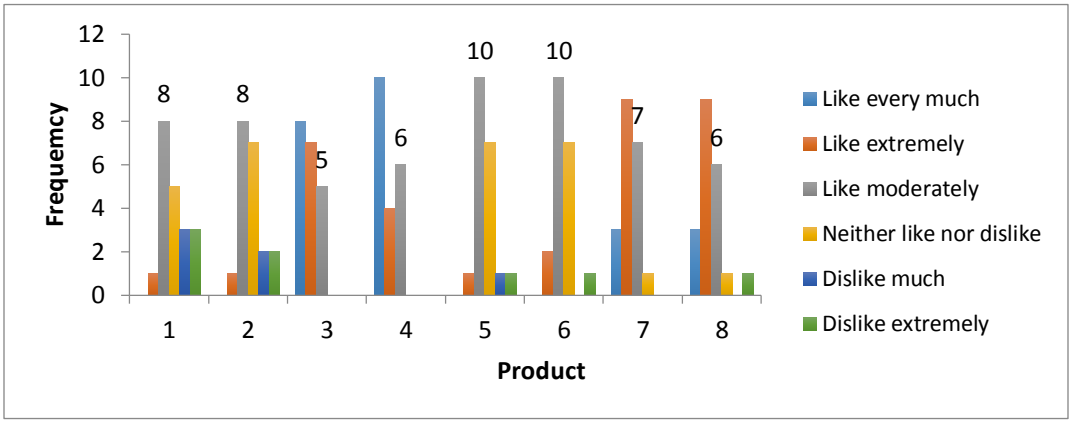

Figure: 4.5. Frequencies of choice made for the Sweetness of the composite bread

4.3.2.4. Bitterness of the composite bread

Regarding the bitter taste of bread, related to the bitter taste in the pulp used in the powder, 45 (28.13%) moderately liked it. The sample with the highest frequency out of those that moderately liked the bitterness, 12 (26.66%) preferred sample 3. This was followed by (11) 24.44% from sample 4 (Fig.4.6).

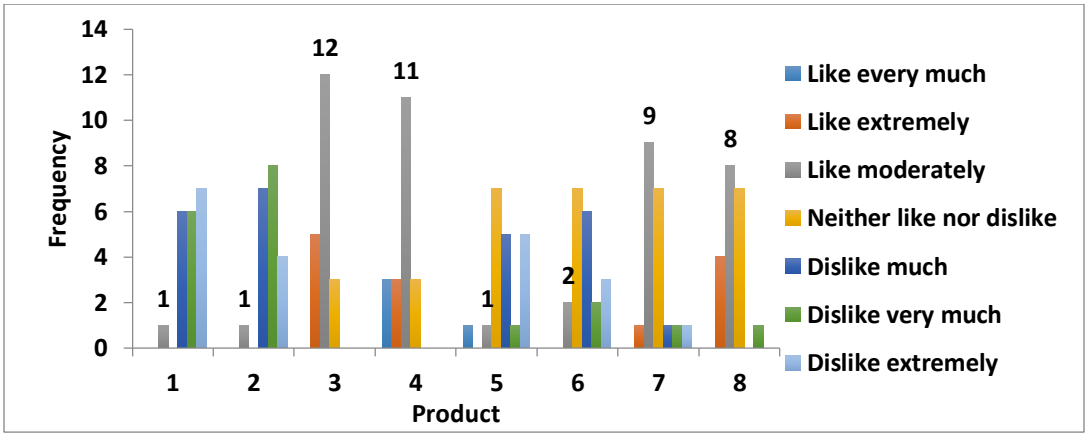

Figure: 4.6. Frequencies of choice made for the bitter taste of the composite bread

4.3.2.5. Savouriness of the composite bread

Of the 160 samples used for savoury taste analysis, majority (78) 48.75%, neither liked nor disliked the bread. Only (2) 1.25% liked it very much. Samples 5 and 6 (14) 17.9% each gained preference over the others with respect to those who neither disliked nor liked the samples (Figure 4.7).

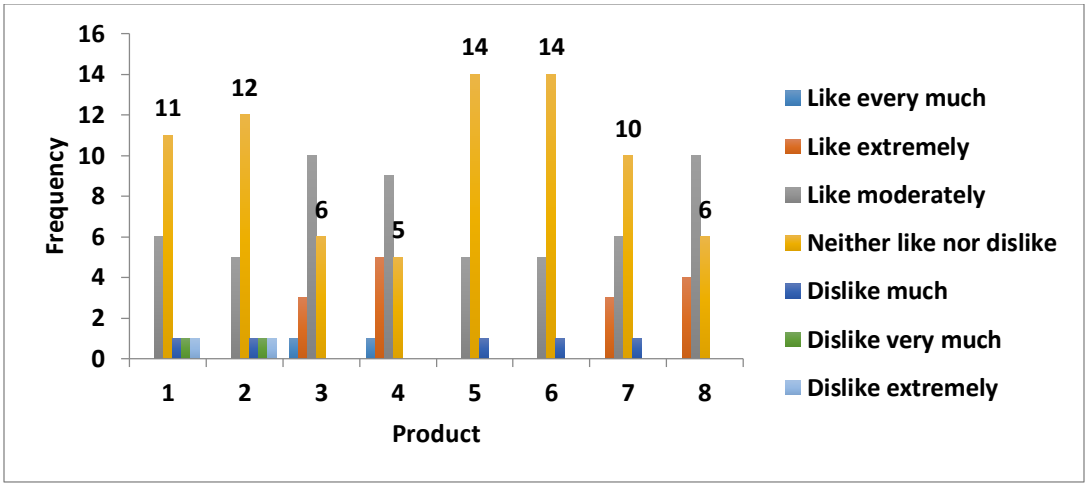

Figure: 4.7. Frequencies of choice made for the Savoury taste of the composite bread

4.3.3. Appearance of the composite bread

Considering the results for appearance of the samples (Figure 4.8), 66 (41.25%) thought the bread was not too attractive. Among these, samples 1 and 2 pooled (18 and 20) 27.3% and 30.3%, respectively to represent the two most preferred.

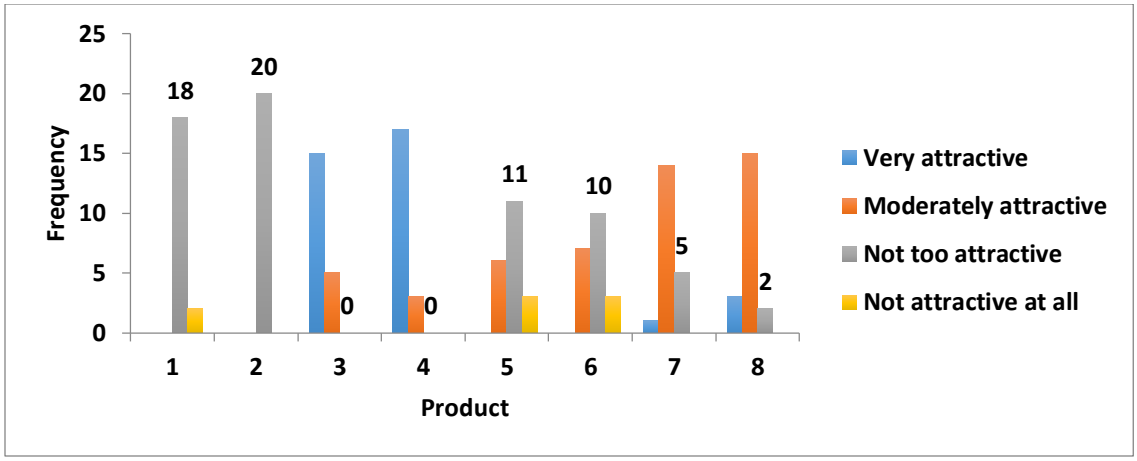

Figure: 4.8. Frequencies of choice made for the appearance of composite bread.

4.3.4. Firmness of the composite bread

Assessing the ability to hold sample, (18) 11.25% agreed it had very good ability, (49) 30.62% good, (66) 49.38% fair whilst (14) 8.75% thought it was poor. Samples 1 and 2 were the best with frequencies of (20, 19) representing 30.30%, and 27.79% respectfully of those that thought the samples had fair firmness (Figure 4.9).

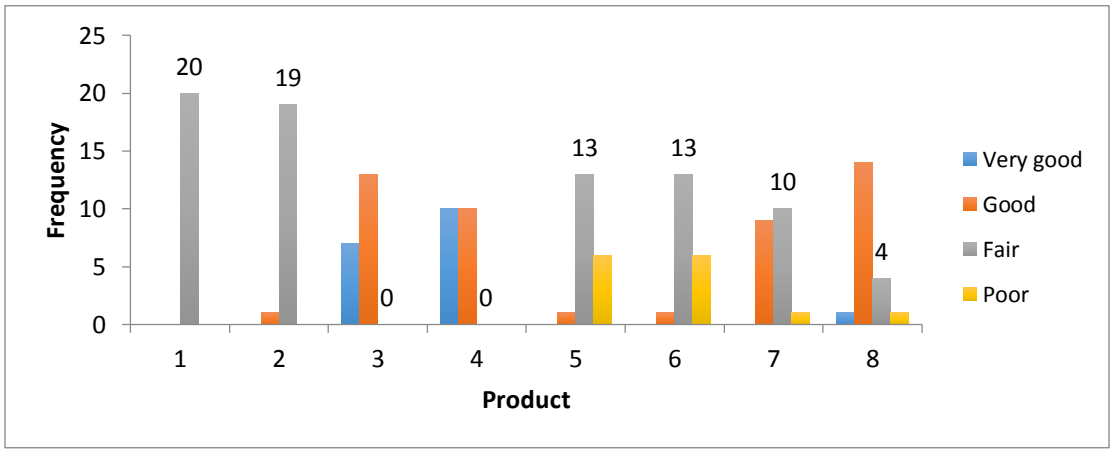

Figure 4.9. Frequencies of choice made for the composite breads Firmness (ability to hold)

4.4. PARTICIPANTS' SOCIO-DEMOGRAPHIC CHARACTERISTICS

A total of 122 participant participated in the study, out of which 63.9% were female and 36.1% male. Majority 59.8% of them were 50 years and above, 89.3% lived within the Accra Metropolis and 67.2% married. Medically, all the participants were diagnosed of having cardiovascular related diseases, but most (51.6%) of them had hypertension (Hpt) alone with 2 (1.6%) of them diagnosed with stroke. Though majority of them had been diagnosed of CVD, their complaints varied. Some of the participants (37.7%), felt some discomfort around the chest, 23% could not sleep well at night, 37.7% experienced palpitations and 31.1% pedal oedema. Majority of the participants (99.1%) had within the past 12 months been having abnormal (high) blood pressure (systolic > 140mmHg or diastolic > 90 mmHg) and on hypertensive medication. Prescribed medications that most of the participants were using include, amlodipine, Lisinopril, Candesartan, Bendrofluazide, Nifidepine, Atorvastatin, Simvastatin, Rosuvastatin in varied dosage. A family history of heart disease (23%), high blood pressure (63.9%), high cholesterol (33.6%) and 28.7% stroke was declared by most participant. With respect to their social life, 95.1% of the participants responded that, they have not used tobacco, however, 9% responded they took a bottle or two of beer or Guinness stout with others going for a spirit or wine. None of the participants has used recreational (street) drugs or treated for mental illness before. At the baseline when the socio-demographic parameters of the intervention and control group were compared, no significant differences were realised with the exception of the male age categorization where p=0.000 (Tables 4.5 and 4.6).

Table 4.5. Frequencies of Demographic Characteristics of Participants

Parameters	Total(122)	Group		P-value
		Control	Participant	
Age (Male)				
19-29	2(1.6)	1(45)	1(45)	**0.000**
30-39	8(6.6)	6(27.3)	2(9.1)	
40-49	7(5.7)	4(18.2)	3(13.6)	
50+	27(22.1)	11(50)	16(72.7)	
Age (Female)				
19-29	2(1.6)	0(0.00)	2(4.9)	0.100
30-39	7(5.7)	4(10.8)	3(7.3)	
40-49	23(18.9)	15(40.5)	8(19.5)	
50+	46(37.7)	18(48.6)	28(68.3)	
Gender				
Female	78(63.9)	37(47.44)	41(52.56)	0.467
Male	44(36.1)	22(50.00)	22(50.00)	
Diagnosis				
Chol, Hpt	32(26.2)	11(33.33)	21(66.67)	
Chol,Hpt,Dm	8(6.6)	2(25.00)	6(75.00)	
Hpt	63(51.6)	37(58.73)	26(41.27)	
Hpt, Dm	9(7.4)	5(55.56)	4(44.44)	
Hpt, Stroke	1(0.8)	0(0.00)	1(100.00)	
Stroke	1(0.8)	0(0.00)	1(100.00)	
Financial Status				
High	6(4.9)	3(50.00)	3(50.00)	0.997
Low	58(47.5)	28(48.28)	30(51.72)	
Middle	58(47.5)	28(48.28)	30(51.72)	
Ethnic group				
Akan	47(38.5)	21(44.68)	26(55.32)	0.031
Dagbane	3(2.5)	2(66.67)	1(33.33)	
Ewe	37(30.3)	16(43.24)	21(56.76)	
Ga	24(19.7)	10(41.67)	14(58.33)	
Others	11(9)	10(90.90)	1(9.10)	
Marital Status				
Deceased	6(4.9)	2(3.4)	4(6.4)	0.245
Divorced	17(13.9)	5(8.5)	12(19.1)	
Married	82(67.2)	43(72.9)	39(61.9)	
Single	16(13.1)	9(15.3)	7(11.1)	

Social History				
Tobacco Users	116(95)	56(48.28)	60(51.72)	0.628
No-Tobacco	6(5)	3(50.00)	3(50.00)	
Drink Alcohol	121(99.2)	58(47.93)	63(52.07)	0.484
no Alcohol	1(0.8)	1(100.00)	0(0.00)	
Occupation				
Bankers	3(2.5)	0(0.0)	3(4.8)	0.135
Pensioners	27(21.1)	11(18.6)	16(25.4)	
Self Employed	18(14.8)	12(22.3)	6(9.5)	
Students	2(1.6)	2(3.4)	0(0.0)	
Traders	29(23.8)	15(25.4)	14(22.2)	
Others	43(35.2)	19(32.2)	24(38.1)	

Data shows frequencies (Percentages) of categorical variables. Chi-square was used to compare categorical data with three or more groupings and Fischer's exact for two groupings. Chol: cholesterol, DM: diabetes mellitus, Hpt: hypertension, Others: pastor, hairdresser, artisan

Table 4.6. Current Complaints and Family History

Parameter	Group		P-value
	Control	Inter	
CURRENT COMPLAINTS			
Chest Pain			
Present	22(37.3)	23(356.5)	1.000
Absent	37(62.7)	40 (63.5)	
Shortness of Breath			
Present	24(40.7)	22(34.9)	0.577
Absent	35(59.3)	41(65.1)	
Dizziness and fainting			
Present	23(39)	15(23.8)	0.081
Absent	36(61)	48(76.2)	
Orthopnea			
Present	11(18.6)	14(22.2)	0.066
Absent	48(81.4)	49(77.8)	
Pedal Oedema			
Present	15(25.4)	23(36.5)	0.241
Absent	44(74.6)	40(63.5)	
Palpitation			
Present	27(45.8)	19(30.2)	0.093
Absent	32(54.2)	44(69.8)	
FAMILY HISTORY			
Heart Disease			
Present	14(24.1)	14(22.2)	0.647
Absent	39(67.2)	46(73)	
Not Sure	5(8.6)	3(4.8)	
High Blood Pressure			
Present	34(57.6)	44(69.8)	0.255
Absent	20(33.9)	13(20.6)	
Not Sure	5(8.5)	6(9.5)	
Cholesterol			
Present	17(28.8)	24(38.1)	0.19
Absent	34(57.6)	26(41.3)	
Not Sure	8(13.6)	13(20.6)	
Stroke			
Present	16(27.1)	19(30.2)	0.636
Absent	39(66.1)	42(66.7)	
Not Sure	4(6.8)	2(3.2)	

Data shows frequencies (Percentages) of categorical variables. Chi-square was used to compare categorical data with three or more groupings and Fischer's exact for two groupings

4.5. ANTHROPOMETRIC CHARACTERISTICS OF PARTICIPANT

Tables 4.7, 4.8 and 4.9 represent the frequencies and means of anthropometric markers of participant in the control and intervention groups of the study. Using the AHA (2017); WHO (2014); NCEP ATP III, (2002) guidelines, the measurements were placed into categories. With regards to visceral fat above the normal category, 65.1% and 57.1% were observed for participants before and after the intervention respectively. Central obesity was prevalent among the females than males in both the participants and control group. However, this did not reduce significantly (p=0.0644) after the intervention. In terms of obesity, (77 05%) were either overweight or obese before the intervention period and no significant difference was realised post intervention. The percentage body fats levels for both male and female between 40-59 years and between both groups were above normal as compared to those above or below this age group. On the whole, with the exception of means of waist circumference, that realised some significant (p= 0.047) reduction between males (90.5±13.6) to (88.4±13.6) and females (102±15.1) to (99.9±13.7) after intervention, no significant reduction in frequencies and means were realised in most of the anthropometric parameters after the intervention. In addition, with regards to the gender of participants and their mean anthropometric measurements, no significant reduction was observed for weight, BMI, visceral fat and metabolic age after the intervention was given. Waist circumference, however significantly (p= 0.047) reduced after the intervention

Table 4.7. Frequencies of Anthropometric characteristics of Participants

	BEFORE intervention			P-value	AFTER intervention			P- value
Parameters	**Total**	**participants**	**control**		**Total**	**Participant**	**Control**	
Visceral Fat								
Normal	48(39.3)	22(34.9)	26(44.1)	1.000	49(40.2)	27(42.9)	22(37.3)	
High	60(49.2)	34(54)	26(44.1)		61(50)	31(49.2)	30(50.8)	
Very High	14(11.5)	7(11.1)	7(11.9)		12(9.8)	5(7.9)	7(11.9)	
W C (M)								
Normal	34(79.1)	17(81)	17(77.3)	1.000	37(86)	19(90.5)	18(81.8)	0.664
C-Obesity	9(20.9)	4(19)	5(22.7)		6(14)	2(9.5)	4(18.2)	
W C (F)								
Normal	13(16.7)	6(14.6)	7(18.9)	0.419	18(23.1)	9(22)	9(24.3)	0.507
C-Obesity	65(83.3)	35(85.4)	30(81.1)		60(76.9)	32(78)	28(75.7)	
BMI								
Underweight	1(0.8)	0(0.0)	1(1.7)	0.572	0(0.0)	0(0.00)	0(0.00)	0.581
Normal	27(22.1)	11(17.5)	16(27.1)		32(26.2)	15(23.8)	17(28.8)	
Overweight	30(24.6)	17(27)	13(22)		27(22.1)	17(27)	10(16.9)	
Obese	48(39.3)	26(41.3)	22(37.3)		48(39.3)	23(36.5)	25(42.4)	
Morbidly Obese	16(13.1)	9(14.3)	7(11.90		15(12.3)	8(12.7)	7(11.9)	

Data shows frequencies (Percentages) of categorical variables. Chi-square was used to compare categorical data with three or more groupings and Fischer's exact for two groupings. BMI: body mass index, WC: waist circumference, M: male, F: female, P-V; p-value, C- Obesity: central obesity

Table 4.8. Anthropometric Frequency of Participants (body fats)

Body fat	Before Intervention			P-value	After Intervention			P-value
	Total	Participant	Control		Total	participant	Control	
Female (19-39yrs)								
Low	0(0)	0(0.0)	0(0.0)	0.217	0(0)	0(0.0)	0(0.0)	0.217
Normal	3(3.8)	1(20)	2(50.0)		3(3.8)	1(20.0)	2(50.0)	
High	1(1.3)	0(0.0)	1(25.0)		1(1.8)	0(0.0)	1(25.0)	
Very high	5(6.4)	4(80.0)	1(25.0)		5(6.4)	4(80.0)	1(25.0)	
Female (40-59yrs)								
Low	0(0)	0(0.0)	0(0.0)	0.585	0(0)	1(2.8)	0(0.0)	0.317
Normal	6(7.7)	2(5.6)	4(12.5)		5(6.4)	1(2.8)	4(12.5)	
High	5(6.4)	3(8.3)	2(6.3)		6(7.7)	4(11.1)	2(6.3)	
Very high	57(73.1)	31(86.1)	26(81.2)		56(71.8)	30(83.3)	26(81.2)	
Male (19-39yrs)								
Low	0(0)	0(0.0)	0(0.0)	1.000	0(0)	0(0.0)	0(0.0)	1.000
Normal	1(2.3)	0(0.0)	1(100)		1(2.3)	0(0.0)	1(100)	
High	0(0)	0(0.0)	0(0.0)		0(0)	0(0.0)	0(0.0)	
Very high	1(2.3)	1(100)	0(0.0)		1(2.3)	1(100)	0(0.0)	
Male (40-59yrs)								
Low	1(2.3)	0(0.0)	1(48)	0.721	2(4.5)	2(9.5)	0(0.0)	0.545
Normal	14(31.8)	7(33.3)	7(33.3)		13(29.5)	6(28.6)	7(33.3)	
High	9(20.5)	4(19)	5(23.8)		10(22.7)	5(23..8)	5(23..8)	
Very high	18(40.9)	10(47.6)	8(38.1)		17(38.6)	8(38.1)	9(42.9)	

Data shows frequencies (Percentages) of categorical variables. Chi-square was used to compare categorical data with three or more groupings and Fischer's exact for two groupings

Table 4.9. The Mean Anthropometric characteristics of Participants

Parameter	Control		P-value	Participants		p-value
	Before intervention	After intervention		Before intervention	After intervention	
Weight						
Male	80.7±23.8	78.4±22.8	0.799	74.3±12.7	73.2±13.3	0.647
Female	82.4±19.9	84.3±20.3		88.0±16.1	87.6±16.4	
W C						
Male	91.2±17.6	89.5±15.1	0.518	90.5±13.6	88.4±13.6	**0.047**
Female	98.6±15.2	98.5±15.4		102.0±15.1	99.9±15.7	
BMI						
Male	27.3±7.1	27.5±6.6	0.399	25.9±3.5	25.3±3.7	0.696
Female	31.7±6.8	32.2±6.7		34.3±6.5	33.4±6.5	
Visceral Fat						
Male	10.0±6.0	10.0±6.0	0.700	10.0±4.0	9.0±4.0	0.654
Female	10.0±3.0	10.0±3.0		11.0±3.0	11.0±2.0	
Metabolic Age						
Male	52.0±20.0	64.0±10.0	0.634	51.0±13.0	50.0±13.0	0.125
Female	63.0±12.0	63.0±12.0		67.0±10.0	52.0±19.0	

Independent t- test was used to compare the means differences of variables. BMI: Body Mass Index, WC: Waist Circumference. P value ≤ 0.05 as significant

4.6. BLOOD PRESSURE TREND AMONG PARTICIPANTS

The prevalence of systolic pressure (using AHA, 2017 guidelines) recorded initially showed that those who had stage 2 hypertension, representing 36.5% of participants, 25.4% stage 1, 13% had levels that needed urgent treatment with 12.7% in desired range before the intervention. There was a significant (p=0.019) reduction between the groups after the interventional period (Table 4.10). The trend was similar with diastolic pressure readings. Initially, 36.5%, of participants had hypertension stage 2, 25 4% stage 1 and 19% needed urgent treatment. After intervention, all the categories reduced significantly (p= 0.001). The systolic (161±22.5) and diastolic (99.2±13.6) means realised significant

reductions with p-values of 0.005 and 0.001 respectfully among participants after the intervention (Table 4:11).

Table 4.10. Systolic and Diastolic Blood Pressure of Participants

	BEFORE intervention			P-value	AFTER intervention			P-value
	Total	**Participant**	**control**		**Total**	**participant**	**control**	
Systolic								
Desired Hypertension	14(11.5)	8(12.7)	6(10.2)		32(26.2)	23(36.5)	9(15.3)	**0.019**
Prehypertension	8(6.6)	3(4.8)	5(8.5)		18(14.8)	12(19)	6(10.2)	
Hypertension Stage 1	33(27)	16(25.4)	17(28.8)	0.883	42(34.4)	17(27)	25(42.4)	
Hypertension Stage 2	44(36.1)	23(36.5)	21(35.6)		20(16.4)	7(11.1)	13(22)	
Hypertensive Urgency	23(18.9)	13(26)	10(16.9)		10(8.2)	4(6.3)	6(10.2)	
Diastolic								
Desired Hypertension	13(10.7)	7(11.1)	6(10.2)		48(39.3)	35(55.6)	13(22)	**0.001**
Prehypertension	9(7.4)	5(7.9)	4(6.8)		12(9.8)	4(6.3)	8(13.6)	
Hypertension Stage 1	38(31.1)	16(25.4)	22(37.3)	0.701	45(36.9)	19(30.2)	26(44.1)	
Hypertension Stage 2	42(34.4)	23(36.5)	19(32.2)		13(10.7)	2(3.2)	11(18.6)	
Hypertensive Urgency	20(16.4)	12(19)	8(13.6)		4(3.3)	3(4.8)	1(1.7)	

Data shows frequencies (Percentages) of categorical variables. Chi-square was used to compare categorical data with three or more groupings and Fischer's exact for two groupings

Table 4.11. Means of Blood Pressure of Participants

	Before Intervention		p-value	After Intervention		p-value
	Participant	**Control**		**Participant**	**Control**	
Systolic	161.2±25.5	156.7±21.4	0.294	137.6±22.9	149.1±21.2	**0.005**
diastolic	99.2±13.6	97.0±12.5	0.351	85.1±10.8	88.7±8.2	**0.009**

Systolic and Diastolic values of Blood Pressure was compared using a P- value ≤ 0.05 to represent significance. Independent t-test compared mean differences.

4.6.1. TREND OF BLOOD PRESSURE READINGS OVER THREE MONTHS

The mean systolic and diastolic blood pressure measured at four different point in the three month period has been presented in Table 4.12. The systolic means recorded between the control and interventional groups reduced significantly (p = 0.005) from the third and fourth readings after receiving the intervention. A similar trend in diastolic means was experienced after the intervention was given. A significance reduction (p=0.002) was again realized in the third and in fourth readings (p=0.009).

Table 4.12. Means of Participants Blood Pressure Readings Over Three Months

		Group		
	Readings	**Control**	**Inter**	**p-value**
Systolic	1	156.7±21.4	161.2±25.5	0.298
	2	150.0±21.4	145.1±22.0	0.218
	3	152.4±20.5	141.0±23.5	**0.005**
	4	149.1±21.1	137.6±22.9	**0.005**
Diastolic	1	97.0±12.5	99.2±13.6	0.351
	2	92.9±10.7	89.4±13.2	0.119
	3	94.0±9.4	87.8±11.7	**0.002**
	4	89.7±8.2	85.1±10.8	**0.009**

Means of Blood Pressure trends used a P value ≤ 0.05 as significance. Independent t- test was used to compare the means differences. All values are in mmHg (mill moles per mercury)

4.7. BIOCHEMICAL CHARACTERISTICS OF PARTICIPANTS

4.7.1. Means of Lipid Parameters within and Between Groups

Table 4.13, shows means ± standard deviation (SD) of biochemical parameters of participant for the control and intervention groups before and after the three months intervention period. The means of total cholesterol (TC), low density lipoproteins cholesterol (LDL-C) and high density lipoproteins cholesterol (HDL-C) recorded significant reductions (TC- p= 0.001, LDL-C- p-= 0.016 and HDL-C p= 0.000) among participants after consumption of the bread. However, no significant reduction was seen in TG within groups before and after intervention.

Table 4.13. Means of Lipid parameters of Participants in general

	Before Intervention		p-value	After Intervention		p-value
Parameter	**Participant**	**Control**		**Participant**	**Control**	
TC	5.8±1.2	5.9±1.1	0.567	4.9±1.1	5.7±1.2	**0.001**
LDL-C	3.4±1.2	3.4±1.1	0.893	2.8±0.9	3.3±1.2	**0.016**
TG	1.1±0.4	1.1±0.4	0.993	1.3±0.5	1.1±0.3	0.177
HDL-C	2.1±0.6	2.2±0.5	0.372	1.5±0.4	1.9±0.4	**0.000**

TC: Total Cholesterol, TG: Triglyceride, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol. P value ≤ 0.05 as significant. The mean differences was compared using independent t-test

4.7.2. Means of Lipid Parameters In Relation To Gender

Table 4.13, shows means ± standard deviation (SD) of biochemical parameters of participant (in relation to the gender) within the two groups, before and after giving the intervention. This was necessary to determine the significance of gender most affected by dyslipidemia and intervention in the study. The means of total cholesterol (TC), recorded significant difference between female and male; (p= 0.016) for control and intervention (p=0.00). Similarly, significant differences for control and

intervention groups were recorded for the HDL-C of participants p=0.0247 and 0.001. However, with LDL-C and TG's, significant differences for LDL-C (p= 0.00) and for TG (p= 0.02) were recorded between male and female in the intervention group.

Table 4.13. Means of Lipid parameters of Participants in Relation To Gender

	Before intervention			After intervention		
Gender	**Participant**	**Control**	**P-Value**	**Participant**	**Control**	**P-value**
	TC			**TC**		
Female	5.7±1.3	6.0±1.1	**0.0160**	5.0±1.2	5.9±1.4	**0.0000**
Male	5.7±1.3	5.9±1.0		4.9±1.1	5.7±1.1	
	LDL			**LDL**		
Female	3.4±1.2	3.5±1.2	0.7045	2.9±0.9	3.5±1.4	**0.0002**
Male	3.3±1.3	3.3±0.9		2.9±0.9	3.3±1.1	
	HDL			**HDL**		
Female	1.9±0.5	2.2±0.4	**0.0247**	1.6±0.4	2.1±0.7	**0.0001**
Male	2.1±0.4	2.2±0.7		1.2±0.4	2.1±0.7	
	TG			**TG**		
Female	1.2±0.3	1.2±0.5	0.3250	1.3±0.6	1.1±0.4	**0.0243**
Male	1.2±0.3	0.9±0.3		1.3±0.6	1.2±0.5	

TC: Total Cholesterol, TG: Triglyceride, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol, P value ≤ 0.05 was used as significance. Independent t- test compared the mean differences. (1): baseline levels, (2): after three month's interventional period.

4.7.3. Frequencies of categories of Lipid Parameters

With reference to the NCEP ATP III (2002) guidelines, frequencies were obtained for all the lipid parameters in the study (Table 4.15 and 4.16). For LDL-C, 22.1% of participants for both control and intervention groups fell within normal category at baseline, with no significant difference (p=0.089) realised after the period of intervention. At baseline, a total of 34.7% male participants and 61.2% female had their HDL-C within normal, irrespective of the intervention given and period; this did not change significantly. Majority (90.2% for control and 84.4% for intervention) of participant's TGs

were normal, this was also not significantly affected by the intervention. However, TCs within groups before and after intervention realised significant (p= 0.03 before and p= 0.002 after) changes in categories of lipid parameters even though only 38.7% (control) and 43.4% (intervention) were within normal range.

Table 4.15. Frequencies of HDL-C of Participants

Parameter	Before Intervention			P-value	After Intervention			P-value
	Total	Participant	Control		Total	Participant	Control	
Male								
Low	1(0.8)	0(0.0)	1(4.5)	0.512	3(2.5)	3(14.3)	0(0.0)	0.108
Normal	42(34.7)	21(100)	21(95.5)		40(33.1)	18(85.7)	22(100)	
Female								
Low	4(3.3)	4(9.8)	0(0.0)	0.071	4(3.3)	4(9.8)	0(0.0)	0.071
Normal	74(61.2)	37(90.2)	37(100)		74(61.2)	37(90.2)	37(100)	

Frequencies of HDL-C using a P value ≤ 0.05 as significant.

Table 4.16. Frequencies of LDL-C, TG and TC of Participants

	Before Intervention			P-value	After Intervention			P-value
	Total	Participant	Control		Total	Participant	Control	
TC								
Normal	35(28.7)	22(34.9)	13(22)	**0.03**	53(43.4)	33(52.4)	20(33.9)	**0.002**
High Optimal	38(31.1)	13(20.6)	25(42.4)		39(32.0)	23(36.5)	16(27.1)	
High	49(40.2)	28(44.4)	21(35.6)		30(24.6)	7(11.1)	23(39)	
TG								
Normal	110(90.2)	57(90.5)	53(89.8)	0.993	103(84.4)	50(79.4)	53(89.8)	0.102
H Optimal	10(8.2)	5(79)	5(85)		15(12.3)	9(14.3)	6(10.2)	
Abnormal	2(1.6)	1(1.6)	1(1.7)		4(3.3)	4(6.3)	0(0.0)	
V.Abnormal	0(0)	0(0.0)	0(0.0)		0(0)	0(0.0)	0(0.0)	
LDL								
Normal	27(22.1)	19(30.2)	9(15.3)	0.068	43(35.2)	25(39.7)	18(30.5)	0.089
N. Normal	29(23.8)	9(14.3)	20(33.9)		28(23)	17(27)	11(18.6)	
H. Optimal	31(25.4)	15(23.8)	16(27.1)		27(22.1)	13(20.6%)	14(23.7%)	
Abnormal	24(19.7)	14(22.2)	10(16.9)		19(15.6)	8(12.7)	11(18.6)	
V Abnormal	10(8.2)	6(9.5)	4(6.8)		5(4.1)	0(0.0)	5(8.5)	

Data shows frequencies (Percentages) of categorical variables. Chi-square was used to compare categorical data with three or more groupings and Fischer's exact two groupings. TC: Total Cholesterol, TG: Triglyceride, LDL-C: Low Density Lipoprotein-Cholesterol, V.abnormal: very abnormal, N. Normal: near normal, H.Optimal: high optimal

4.8. RELATIONSHIP BETWEEN ANTHROPOMETRIES AND LIPID PROFILE OF PARTICIPANTS BEFORE INTERVENTION

4.8.1. Relationship between Anthropometries and Lipid Profile of Female Participants before and after Intervention

Table 4.17 shows the relationship among waist circumference (WC), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol

(HDL-C), systolic and diastolic parameters before intervention A significant and strong positive relationship (r=0.863, P = 0.000) exist between systolic and diastolic as well as between LDL-C and TC (r=0.853, p=0.00). These were among female participants in the study.

After a strong positive relationship between TC and LDL-C (r= 0.930; p-value 0.000) was recorded. No relationship was recorded between WC and the other parameters as with correlations before intervention.

Table 4.17. Correlation between Anthropometries and Lipid Profile of Female Participants before intervention

Before intervention			
	Indicators	**R**	**p-value**
WC			
	LDL-C	0.239*	0.035
Systolic	Diastolic	.836**	0.000
	TG	0.225*	0.047
TC	LDL-C	0.853**	0.000
LDL-C	HDL	-0.224*	0.048
After intervention			
Systolic	Diastolic	0.282*	0.013
TC	LDL-C	0.930**	0.000
	HDL-C	0.460**	0.000
	TG	0.313**	0.005
LDL-C	HDL-C	0.234*	0.040
	TG	0.240*	0.034
HDL-C	TG	-0.235	0.038

Controlling variables: TC: total cholesterol, TG: triglycerides, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoproteins cholesterol, systolic and diastolic blood pressure. ** Correlation was significant at 0.01 level (2-tailed) and * Correlation was significant at 0.05 level (2-tailed)

4.8.2. Relationship between Anthropometries and Lipid Profile of Male Participants before and After Intervention

Parameters used for correlations among female participants were repeated with males. Before the intervention, systolic and diastolic, total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) realised significant strong positive relationship (r=0.748; p=0.000, r=0.796; p = 0.000) respectfully. These did not change after the intervention was given. In addition, TC and HDL-C realised strong relationship (r=0.526 p = 0.000) after the intervention. Details are presented in Table 4.18.

Table 4.18. Correlation between Anthropometries and Lipid Profile of male Participants before intervention

Before intervention	**Indicators**	**R**	**p-value**
Systolic	Diastolic	0.748**	0.000
TC	LDL-C	0.796**	0.000
Post intervention			
WC			
	TC	0.355*	0.019
	LDL-C	0.382*	0.011
Systolic	Diastolic	0.725**	0.000
	HDL-C	0.359	0.015
Diastolic	TC	0.352*	0.020
	HLD-C	0.470**	0.001
TC	LDL-C	0.900**	0.000
	HDL-C	0.526**	0.000
	TG	0.317*	0.038

Controlling variables: TC, TG, LDL-C, HDL-C, systolic and diastolic blood pressure. Correlation is significant at 0.05 (2-tailed)

4.8.3. Relationship between various anthropometric and Biochemical markers for Intervention and Non-intervention group.

Pearson correlation for different anthropometric and biochemical measures controlling for gender for the intervention and non-intervention groups is presented in Table 4.19. For anthropometries and intervention group, body mass index (BMI) significantly correlated positively with weight (0.814) and WC (0.787), respectively. In addition, waist circumference (WC) significantly correlated positively (0.796) with weight, visceral fats with weight (0.725), WC (0.668) and BMI (0.688) at p- value < 0.01. For biochemical, low density lipoproteins significantly correlated positively (0.919) with total cholesterol.

Table 4.19: Pearson correlation coefficient between Anthropometric and Biochemistry after Intervention (Upper right hand side) and baseline (Lower left hand sided).

	Wt	**Ht**	**WC**	**BMI**	**VF**	**Sys**	**Dia**	**TC**	**LDL**	**HDL**	**TG**
Wt		.064	**.796****	**.814****	**.725****	-.013	.082	.077	.114	-.005	.014
Ht	0.115		-.092	-.103	-.092	-.007	.095	-.018	-.050	.034	.070
WC	**.762****	-0.15		**.787****	**.668****	-.020	.126	.215*	.236**	.052	.151
BMI	**.879****	-.289**	**.792****		**.688****	.085	.219*	.116	.129	.075	.027
VF	**.747****	0.028	**.681****	**.707****		.069	.146	.262**	.305**	.038	.151
Sys	0.098	-0.043	0.108	0.119	0.089		.293**	-.025	.010	.030	-.069
Dia	0.05	-0.06	0.062	0.091	0.036	**.804****		.239**	.212*	.267**	-.077
TC	0.121	-0.047	.201*	0.11	.251**	-0.104	-0.132		**.919****	.482**	.315**
LDL	0.138	-0.058	.222*	0.141	.269**	-0.103	-0.174	**.841****		.231*	.254**
HDL	0.027	-0.02	-0.005	0.005	-0.04	0.011	0.08	.218*	-.205*		-.216*
TG	0.001	-0.171	0.09	0.048	0.132	0.021	0.067	.328**	.225*	0.025	

** Correlation is significant at 0.01 level (2-tailed) and * Correlation is significant at 0.05 level (2-tailed). Wt; weight, Ht; Height, WC; Waist Circumference, BMI; Body Mass Index, VF; Visceral Fat, Sys; Systolic, Dia; Diastolic, TC; Total Cholesterol, LDL; Low Density Lipoprotein, HDL; High Density Lipoprotein and TG; Triglycerides.

4.9. PARTICIPANTS ON MEDICATION: FREQUENCY AND EFFECT ON *BORASSUS AETHIOPUM* COMPOSITE BREAD ON THEIR LIPID PROFILE

4.9.1. Frequencies of Participants on Medication

The Tables 4.20-22, show results of participants on medication, effect of drug on participants and relationship among the various types of drugs (statins). Forty- six participants were on three different medications with varied dosage. Of these participants, 13(28.3%) were male, 33(71.7%) female, 18(39.1%) control and 28(60.9%) intervention group. Considering the medication (statins) participant took, 15(32.6%) were on atorvastatin 10 mg, 7(38.9%) in the control group and 8(28.6%) intervention. 11(23.9%) were on atorvastatin 20 mg, among them, 4(22.2%) were in the control and 7(25%) interventional group. Also, 12(26.1%) participants were on Crestor (rosuvastatin) 10 mg, 6 each (33.3% and 21.4%) belonged to the control and intervention group respectfully. Another, 6(13%) of participants were on Crestor 20mg, 1(5.6%) in the control group and 5(17.9%) intervention. Only 2(7.1) took simvastatin 40mg, all in the intervention group. No significant difference (p= 0.47) was recorded among groups of participant on the various medications.

Table 4.20. Frequency Of Gender Of Participants On Medication

Male	Female
13(28.3%)	33(71.7%)

Group frequencies of participant on medication; using a P value ≤ 0.05 as significant.

Table 4.21. Groupings For Participants On Medication

Control	Inter
18(39.1%)	28(60.9%)

Group frequencies of participant on medication; using a P value ≤ 0.05 as significant.

Table: 4.22. Frequencies on Type of Medication Participants Took

Drug	Group		Total	P-value
	Participant	Control		
Atovas 10mg	8(28.6)	7(38.9)	15(32.6)	
Atovas 20mg	7(25)	4(22.2)	11(23.9)	
Crestor 10mg	6(21.4)	6(33.3)	12(26.1)	0.471
Crestor 20mg	5(17.9)	1(5.6)	6(13)	
Simvas 40mg	2(7.1)	0(0)	2(4.3)	

Frequencies of type of medication; using a P value ≤ 0.05 as significant. Simvas: simvastatin, Atovas: atorvastatin.

4.9.2. Effect of *Borassus aethiopum* Composite Bread on Lipid Profile of Participants on Medication

Assessing the effect of the drug on blood pressure and biochemical parameters, no significant change was recorded among parameters and between the groups. However some significant reductions were realised within the interventional group after consuming the *Borassus aethiopum* composite bread. Systolic (p= 0.005) diastolic (p=0.0002) pressures as well as HDL-C (p=0.0039) improved significant, however, total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) realised no significant changes (Figure 4.23).

With respect to differences in type of medication and dose, significant difference was seen between simvastatin and Crestor 10 mg (p=0.013), simvastatin and atorvastatin 10 mg (p= 0.0184).

Table 4.23. Blood Pressure and Lipid Profile Of Participants on Medication

	Before Intervention			After Intervention		
Indicator	**Participant**	**Control**	**P-Value**	**Participant**	**Control**	**P-Value**
Systolic	160.21	152.5	0.820	135.25	158.17	**0.005**
Diastolic	99.25	92.22	0.784	83.04	98.05	**0.0002**
TC	6.01	5.70	0.956	5.02	6.03	0.697
LDL-C	3.8	3.60	0.328	2.03	3.38	**0.048**
HDL-C	2.02	1.00	0.384	1.41	2.2	**0.0039**
TG	1.11	1.17	0.273	1.34	0.99	0.3623

TC= Total Cholesterol, LDL-C = Low Density Lipoprotein Cholesterol, HDL-C = High Density Lipoprotein Cholesterol, TG = Triglycerides

CHAPTER FIVE

5.0. DISCUSSION

A total of 122 participant participated in the study, this includes 63.9% female and 36.1% male with majority 59.8% of them 50 years and above. This implies that, cardiovascular diseases (CVD) are prevalent and widespread among females as compared to male, consistent with a study by Ford *et al*., (2007), where females outnumbered males with respect to CVD. Again in a survey conducted by the M Ghana Demographics Profile, (2017), more female attended hospital for various reasons than the males. This accounts for the increase in female recruitment in the study than males. The prevalence of CVD according to Ford *et al.* (2007), is proportional to age. This is consistent with this study as majority of female participants 37.7% and 22.13% males were over fifty years. With respect to the cause and risk factors of CVD's, it is noted that, hypertension, dyslipidemia, diabetes mellitus, smoking and tobacco use could increase ones chance of developing the disease (Chow, 2008; Addo *et al*., 2012, Micah and Nkum, 2012; Lloyd-Jones *et al*.,2010). A family history of hypertension (HPT), dyslipidaemia and diabetes mellitus (DM) was realised in 27% of participants as some of the predisposing factors, this is consistent with studies done on CVD's and one by Appannah *et al.*, (2015), when they recorded cardio metabolic determinates and pro-atherogenic factors such as obesity, dyslipidaemia and hyperglycaemia to been linked to CVD's. In addition, 9% of the participants recruited in the study took alcohol and 4.9% used tobacco. Despite the fact that the study realised these frequencies, Martin-Timon *et al.* (2014), and Asfaw, (2005) identified smoking and the use of tobacco as predisposing factors for the development of CVD in individual with or without family history of CVD.

With the increased prevalence of CVD in Ghana and its related causes, Asfaw (2005), realised that, due to changes in diet (increase in polished foods and fast foods consumption, reduction in vegetable consumption as well as less fibre foods), obesity is on the increase, increasing CVD risk. In Ghana, the consumption of fruits and vegetables is poor irrespective of its availability. Some studies on *Borassus*

aethiopum fruit (pulp) established the fact that, the plant is available in Ghana, the pulp used as food either eaten raw or used as an ingredient in food preparation (Ali *et al*.,2010b; Gruca *et al.*, 2015). They also established the fact that, the fruit was not only suitable as food but also possessed pharmacological properties. Despite the properties found in the fruit of the Palmyra plants and the fact than it is often not sold, the woody tough nature of the fruit renders it difficult to be incorporated into the daily diet of most people. The processing of the fruit into flour and further into composite bread was handy and rendered its use effectively. After the anthropometries, the *Borassus aethiopum* composite bread was developed and its microbial content assessed.

Total aerobic count is relevant in establishing the degree of contamination of samples. The results obtained did indicate the presence of some aerobic microorganisms in the flour. The detected counts were within acceptable limit and this is ideal for the acceptability and safety of the product. The cooking process was expected to reduce the population of these aerobic contaminants to acceptable numbers (Dickens, *et al*., 1994). The milling process involving the hammer mill produced a great amount of heat which could have killed some of the microorganisms coupled with the stress and impact forces of the piston on the flour. Microbial growth are influenced by moisture, mass, time, temperature and surface area (Gillooly, *et al*., 2001), thus if these conditions are not optimized the rate and efficiency of the growth is affected.

The moisture content of the flour was relatively low, since it was solar dried, the microbes were exposed to exposes the Ultra violet rays of the sun, known to denature these microorganisms. The result for the bread even though slightly higher than the powder was still within the safe limits. The public industrial mixer used, cooling method and handling, may account for the increase in microorganism.

Coliforms are a class of organisms which are of prime importance in the area of food safety as they have been implicated in diarrhoea, stomach cramps, vomiting and contamination cases recorded across the globe. This makes Coliforms pathogenicity of concern to health and safety of consumers. The

family of Coliforms consists of organisms such as *Klebsiella pneumoniae* which causes pneumonia, *Enterococci* spp., and *Escherichia coli* responsible for diarrhoea experienced from food contamination (Thiruvengadam *et al*., 1973).

Coliforms in foods pose several health issues hence standards ranging from 0 to 10 cfu/g as the tolerable limits, however in Ghana, the coliform tolerable limit is zero (0). This is as a result of the poor hygiene and sanitary conditions of the immediate environment most items are prepared or sold.

The results of this study generally showed no coliforms detected in the samples. This made the product safe for consumption with no risks of complications aftermath, regarding coliform induced infections. One typical coliform that is of significance in food safety is *Escherichia coli* and is mostly used as the indicator for faecal contamination and human induced contamination. The detection of *E. coli* strains particularly the pathogenic strains in food is deemed a high alert factor, thus the acceptable or tolerable limit of *E. coli* being set at 0 cfu/g by the ISO and AOAC. No *Escherichia coli* were also detected in the samples. This points to or indicates the hygienic state of the manufacturing process or line used in the development of the product.

The presence of yeast in foods is mostly an indicator of a fermentation process which could be ideal but in other cases an indicator of spoilage. Yeast mostly develop in foods over prolonged storage thus the detection of insignificant yeast presence points to the freshness of the samples and also gives an index of the quality of the samples.

The anthropometric measurement used in this study to determine obesity levels and cardiovascular risk were, BMI, waist circumference (WC), body fat and visceral fat before and after the intervention. De – Koning (2007), in a study, established the use of these indicators individually or with others for effective results and its interpretation. According to Appannah *et al.,* (2015) cardio metabolic determinates and pro-atherogenic factors such as obesity, dyslipidaemia and hyperglycaemia have been linked to CVDs. These can be determined by the use of several anthropometric measurement and

biochemical markers. Artherogenecity the pathogenesis of CVD, is strongly linked to dyslipidaemia hence body fats (Liu *et al*., 2011). This means that the amount of body fat predisposes an individual to cardiovascular risk or its progression (Wormser et al., 2011). Female participant of this study had higher body fats 55.7% than males 22.9%. Mathieu *et al*. (2009), attributed this difference to the gynoid physique of female as compared to male. The reduction of body fats after the intervention was not significant. According to Pashkow (2011), different mechanism other than diet is required for significant reduction in body fats to be realised. This probably accounts for the reason why body fats in participants did not see any significant reduction after the intervention.

In addition to the above, BMI was used to indicates the various levels of obesity. Zheng *et al*. (2011) and De-Koning (2007), expressed obese individuals as being predisposed to adipose accumulation correlated to high risk of stroke. However, a reduction in levels was seen after intervention. This reduction can be attributed to the saponins in the *Borassus aethiopum* composite bread. These saponins in the bread facilitates excretion (quantity and frequency) ultimately leading to reduction in weight. With regards to obesity, the use of BMI only examines weight associated with muscles than fats and its distribution in the body, (Vinik 2005), necessitating the use of other indicators in addition to BMI to establish the type of obesity in participants.

Visceral fat measurement was used in assessing further the distribution of fat in the body and further establishes the level of CVD risk and its progression among participants. With regards to body fat distribution and cardiovascular risk, Rosito *et al*. (2008), in their study linked abdominal adiposity to CVD, adding that a strong correlation existed between the two. The results of this study showed that, participants showed varied levels, normal and high before and after the intervention. Even though there was some amount of reduction realised, most of the participants still had increased visceral fats. This could be attributed to the age of participant. Ford *et al*. (2007), indicated that visceral fat had a linear correlation with atherosclerosis. The higher an individual advances in age, the greater the chances of

accumulating visceral fats (Mattieu *et al*., 2009). It could also be argued that, the duration for the intervention was limited hence the reduction of visceral fats after three months not being significant. Furthermore, the use of waist circumference (WC) in determining accumulation of abdominal adiposity has been shown in several studies to be beneficial. Unlike visceral fat and BMI, guidelines used in establishing individual's levels vary with gender (WHO 2014). Freibeng *et al*. (2008), realised that, abdominal girth established for male and female was necessary since both had different body shape. This renders its use more effective and precise than BMI. Levels of WC realised in this study for central obesity in males were 53.3% and females were 73%. However, after the intervention, this rose to 77.1% in males and 83.3% in females. Mathieu *et al*. (2009), also realised a high prevalence of central obesity among the female populace but attributed it to their gynoid physique. The high levels observed in this study indicates high deposition of abdominal fats which according to Korhonen *et al.* (2009); Freibeng *et al*. (2008) and Arambepola *et al*. (2008), increases blood pressure; levels consistent with systolic and diastolic levels observed in this study. The initial means of 156.7±21.4, 161.2±25 was realized for systolic and 97±12.5/ 99.3±13.7 for diastolic before intervention for both intervention and control groups respectively. During the period of intervention, blood pressures saw a significant ($p=0.005$) reduction from the second month in the interventional period, systolic means recorded a ($p= 0.002$) and ($p= 0.009$) for diastolic. Reductions in frequencies and improvement in levels of the various indicators after intervention validates the positive effect of the constituents in the *Borassus aethiopum* composite bread. These reductions according to Fox *et al.* (2007), will attenuates the artherogenic effect and secretion of lipid accumulation enzymes and over activation of enzymes responsible to insulin metabolism in CVD patients Korhonen *et al.* (2009).

The pathogenesis of CVD according to many studies involves many mechanisms all geared towards the development of atherosclerosis in human vasculature (Greaves and Gordon, 2009). In this study, all participants were screened for their serum lipids; total cholesterol (TC), triglycerides (TG), low density

lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) to ascertain the levels before and after intervention (*Borassus aethiopum* composite bread).This was necessary to evaluate the effect of the *Borassus* bread on the lipid profile post intervention and make valid conclusions on its anti-lipidemic properties. Dyslipidaemia, one of the causes of CVD was prevalent among participant of this study. In line with the findings of this study, Pashkow (2011), identified dyslipidaemia as one of the main causes of oxidative stress and atherosclerosis that leads to CVD. The NCEP ATP 111 (2002) guidelines, used in categorising the dyslipidemia among the participants, showed that only 28.7% and 46.7% of the participants had their TC and LDL-C within normal levels for both male and female. However, 90.1% had their TG within normal levels. In addition, 97.6% and 94.8% had normal HDL-C levels for both males and females. Several studies have also identified an increase in LDL-C levels in both male and female diagnosed as CVD patients (Niroumand *et al*., 2015; Essah *et al*., 2008; Ogden *et al*., 2016). Appannal *et al.,* (2015) noted that these could be attributed to the multifactorial mechanism of atherosclerosis pathogenesis. Several methods; pharmacology, lifestyle changes and diet among others have been implicated in the reduction or inhibition of these mechanisms, hence a reduction in CVD risk factors and increased progression (Appannal *et al.* 2015).

The *Borassus* composite bread with respect to previous studies on *Borassus* was used in this study to evaluate its pharmacologic effect on CVD patients. After daily consumption of a roll of the *Borassus* bread for three months, the results of lipid parameters realised reductions in levels. The number of participants that had normal levels of TC improved from 28.7% to 43.4%. Their LDL-C also reduced from 22.1% to 35.2%.These differences were realised among those that ate the *Borassus* bread. Additionally, the number of participants initially in the high and abnormal categories also saw a reduction from 40.2% to 24.6% in TC and 53 3% to 49.2% in LDL-C again within the three months period of eating the *Borassus aethiopum* composite bread. These improvements can be attributed to the effect of the *Borassus aethiopum* composite bread on participant's serum lipids. Ali *et al*., (2010);

Vijayakumari *et al*. (2014, 2015), identified several essential phytochemicals (phenol, saponins, terpenes, sterols and steroids, cardiac glycosides and flavonoids) in *Borassus aethiopum* fruit pulp that could be responsible for the reduction. Ali *et a*l., (2010) noted that, these phytochemicals demonstrate antioxidant, anti-inflammatory and anti- atherogenic properties. Relating the mechanism of atherosclerosis to the phytochemicals in the *Borassus aethiopum* composite bread, flavonoids, appreciably present in *Borassus,* attenuate the development of reactive oxygen species (ROS), preventing oxidative, plague aggregation and rapture, halting atherosclerosis formation and build up damage (Dalgard *et al.* 2009; Surmi and Hasty 2008 and Pearson *et al*., 2002). Once these processes are taken care of by the flavonoids, a reduction in artherosclerosis would be experienced hence CVD. The cardiac glycoside present in the bread also acts on the cardiac muscles and in the Na^{+} K lag hypothesis inhibiting Na^{+} K- ATPhase in the body (Gomez *et al.* 1996). Again according to Yu *et al.* (2011), Saponins, due to the membrano-lytic properties it processes, form micelles with bile salt and influence the absorption of lipids by aiding in the excretion of these lipids via the anus (Cheek, 2001). The action of cholestyramine is mimicked by the saponins to again aid in faecal excretion of lipids in the body. Indeed the development of oxidative stress as well as oxidation of LDL's cannot be over emphasised in cardiovascular diseases. In view of this, the AOAC of *Borassus aethiopum* composite bread was examined in this study. The anti-oxidant content of *Borassus* (2.2±0.41) was ten times lesser than that of standard ascorbic acid (0.124±0.02). Anti- oxidant therapy currently represents a promising way for medicine. This renders the bread an important food source in relation to atherogenesis. This study realised that 37.7% of participants were on varied lipid- lowering medications. It could be argued that the lipid- lowing medications (statins) caused the desired results realised. This however, may not be the case, among those on medication 60.9% and who ate the *Borassus aethiopum* composite bread and 39.1% blinded placebo. Those who received the intervention realised reduction in LDL-C, HDL-C, systolic and diastolic blood pressure as compared with those on the blinded placebo. No significant

reduction was realised for TC (0.820), LDL-C (0.328) and TG (0.273). This could be the justification as to why the reduction in lipid profile cannot be linked to the various medications. The presence of phytochemical in the Borassus bread effected the change considering the fact that, before the intervention participants of both groups were all on medication. Again, even after the intervention it was only participants who ate the *Borassus* bread that realised significant reductions or improvement in levels of dyslipidemia. Pashkow, (2011) and De-Marchi *et al.* (2012), identified the need to use combined therapy in treating CVD. They thought the use of diet, exercise and medication could better lower lipids in hyperlipidaemia individuals. Consistent to their study, a combination of the Borassus bread and the medications could be responsible for the reduction in lipids realised in this study. As to the type of statin (atorvastatin, simvastatin, rosuvastatin) participants were on, no significant difference was seen among the various drugs and lipid reduction. This again concludes that irrespective of the type of statin participants were on the action of phytochemical present in the bread, the desired results was equal across board. This was however different when the medications assessed to determine any difference in type and dose realised significant reduction among those on simvastatin as against those on Crestor 10mg and atorvastatin 10mg.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

This study was aimed at determining the phytochemical composition of *Borassus aethiopum* fruit pulp (powder) and explore the use of *Borassus aethiopum* as composite flour in bread making. The phytochemical, antioxidants and microbial load of the bread was determined. The effect of *Borassus aethiopum* composite bread in lowering blood lipid (cholesterol) in hyper-lipidemic individuals was assessed. The following are the summary of results obtained.

- Phytochemicals found in the *Borassus aethiopum* fruit pulp were, flavonoids, saponins, cardiac glycosides, phenols, alkaloids, treterpenes, sterols and steroids. .
- The pulp from the African Palmyra plant (*Borassus aethiopum*) fruit was solar dried, milled and used as flour for confectionery product (bread).
- The micro-organisms in the powdered *Borassus* pulp were within tolerable limits rendering it safe for consumption
- The *Borassus aethiopum* composite bread, made from the powdered pulp of the African Palmyra plant (*Borassus aethiopum*) contained phytochemicals such as flavonoids, saponins, cardiac glycosides, phenols, alkaloids, treterpenes, sterols and steroids.
- Central obesity and dyslipidemia was prevalent among females aged 50 years and above than males of same age
- The Borassus bread intervention, reduced TC by 14.7% and LDL-C by 12.1%
- The participants recorded these reductions after the intervention;

  TC- 5.8±1.2 before and 4.6±1.2 after

  LDL-C 3.4±1.2 before and 2.8±0.9 after

- There was a reduction in LDL-C, HDL-C, systolic and diastolic blood pressures in participants on medication and in the intervention group than the control group.
- Therefore the study revealed that intervention with *Borassus aethiopum* bread, prepared from the African Palmyra plant has lipid lowering properties that can add to the body of evidence in the management of cardiovascular diseases.
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6.2. RECOMMENDATIONS

Further studies need to be conducted to determine the quantities of the various phytochemical in the pulp of the fruit as well as the product developed from it. Further studies will have to be conducted to assess the impact of *Borassus aethiopum* on other disease conditions. The study could also be up scaled in other facilities for a longer period and further toxicity studies done on the various products developed thereafter.

6.3. LIMITATIONS OF STUDY

One of the limiting factors for this study was that the intervention lasted for three months which probably could have been extended beyond the three months but for time and resource constraints. Most of the laboratory investigations were expensive not withstanding the challenges of delayed orders for reagent to be used for the various analyses and this also limited the duration of the intervention. Quantitative analysis of some of the phytochemical could not be done due to lack of protocol to be used in its determination.

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APPENDIX

Preparation of Mannitol Salt Agar

Agar powder (111 g) was suspended in 1 liter of distilled water and brought to boil to dissolve completely. It was sterilized by autoclaving at 121^0 C for 15 minutes and brought to cool at 50^0 C prior to pouring.

Preparation of Malt extract agar

The fungi assay featuring yeast and mould was carried out on malt extract agar prepared by dissolving a gram of the agar powder in a liter of distilled water and brought to boil at 100^0 C for 15 minutes. The media was sterilized at 121^0 C for 15 minutes and brought to cool at 50^0 C prior to plate pouring followed by a 24 hour sterility check

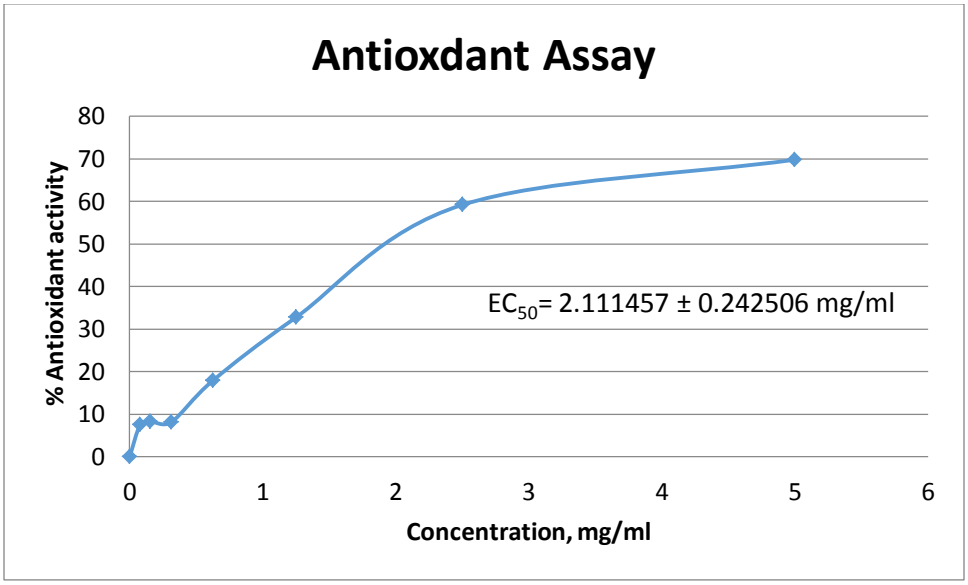

Figure 4.1: Antioxidant activity of *Borassus aethiopum* powder

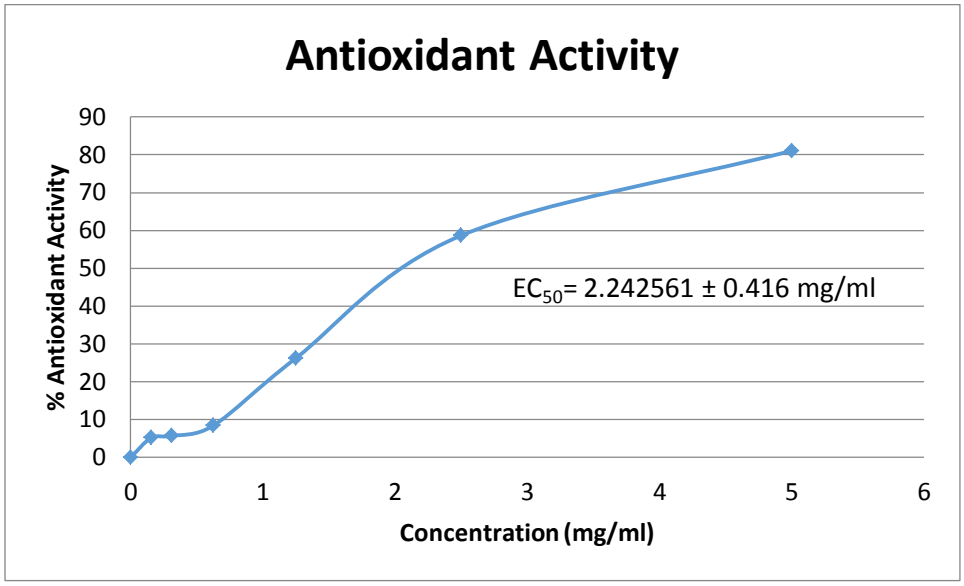

Figure 4.2: Antioxidant activity of *Borassus aethiopum* bread

RESEARCH QUESTIONNAIRE

DEPARTMENT OF BIOCHEMISTY

(KNUST)

RESEARCHER: MARIAN PEPRAH

SUPERVISORS: DR. CHARLES APPREY

PROF. V.P. DZOGBEFIA

INTRODUCTION

The researcher is a final year nutrition student of the Kwame Nkrumah University of Science and Technology.

I seek to evaluate the impact of *Borassus aethiopum* "oman kube" on the lipid profile of individuals with cardiovascular disease. This is a fulfillment of the requirement leading to the award of Masters in human nutrition and dietetics. All information provided for this research would be treated with extreme confidentiality and use for academic purpose only. I'm pleading with you to spare me some few minutes in filling out this questionnaire. Kindly be as sincere as possible.

Thank you.

SECTION A

DEMOGRAPHIC DATA OF RESPONDENT.

PERSONAL HISTORY

Please tick by the suitable answer Code…… (To be filled by researcher)

1. **GENDER**

   Male ☐ Female ☐

2. **DATE OF BIRTH ……..**

3. **AGE**

   19-29 ☐ 30-39 ☐ 40-49 ☐ 50+ ☐

4. **OCCUPATION**

   Student ☐ Banker ☐ Artisan ☐ Trader ☐ Self-employed ☐ others…….....

5. **ETHNIC BACKGROUND**

   Akan ☐ Ewe ☐ Ga ☐ Dagbane ☐ others (specify)…….

6. **PLACE OF RESIDENCE**

   Accra ☐ Tema ☐ outside Accra ☐

7. **FINANCIAL STATUS**

   High income ☐ Middle income ☐ Low income ☐

8. **MARITAL STATUS**

   Married ☐ single ☐ divorced ☐

9. TELEPHONE NO…….

SECTION B CURRENT MEDICAL HISTORY

Please answer by **circling "YES" or" NO"** to the following questions **(10 – 23)**

Do you currently have any of the following?

No	Question	Yes	no
10	**Pain and discomfort in the chest or surrounding area that occurs when you engage in physical activity**		
11	**Shortness of breath**		
12	**Unexplained dizziness and fainting**		
13	**Difficulty breathing at night except in upright position**		
14	**Swelling of the ankles (recurrent and unrelated to injury**		
15	**Heart palpitations (on more than one occasion**		
16	**Pain in the leg that causes you to stop walking**		
17	**Known heart murmur**		
18	**Have you had high or abnormal blood cholesterol within the past 12 months**		
19	**Are you taking medication to control your lipids**		

20 **Within the past 12 months has a health professional told you that you have high blood pressure (systolic > 140 or diastolic > 90**

21 **Currently, do you have high blood pressure**

22 **Within the past 12 months, have you taken any medicine to control your blood pressure**

23 **Have you ever been told by a health professional that you have a fasting blood glucose greater than normal**

SECTION C

PAST MEDICAL HISTORY

PLEASE REVIEW THE FOLLOWING LIST. IF YOU HAVE ANY OF THESE CONDITIONS **Check (√) Yes or No** and year of diagnosis. If you have other conditions not listed, please write them down in the space provided.

	Condition / disease	Yes	no	From – To
24	**Alcoholism**			
25	**Anemia**			
26	**Arthritis**			
27	**Asthma**			
28	**Bone pain**			
29	**Seizures**			
30	**Diabetes mellitus**			

31 **Glaucoma**

32 **Heart disease**

33 **Stroke**

34 **High blood pressure**

35 **Jaundice**

36 **Allergies**

37 **Ulcers/ stomach pain**

38 **Cancer**

39. If you have any other medical problems or serious injuries that are not listed above, please describe them here:

……..

……

40. Please list any surgeries or hospital stays you have had and their approximate date/year:

……

……

……

……

41. **Please list all medications, including vitamins, herbal or natural supplements and prescription medications, which you are currently taking. Please note the dosage if possible.**

Medication		Dosage
Example; Crestor	**10mg**	**1 daily**

SECTION D

FAMILY HISTORY

Check if any of the diseases run in your extended or nuclear family. Please TICK yes, no or not sure

Disease	Yes	no	not sure
42. Alcoholism or Drug Use			
43. Diabetes mellitus			
44. Heart Disease			
45. High Blood Pressure			
46. High Cholesterol			
47. Mental Illness			
48. Stroke			

SECTION E

SOCIAL HISTORY

49. Do you use tobacco products? …….. Yes No

50. Cigarettes (number of packs or sticks in a day) ……

51. Do you use drugs or alcohol? …….. Yes No

52. If you drink alcohol products, what do you drink?.....…….

53. How many bottles or glasses per day?…….……

54. If you use recreational (street) drugs, what do you use and how often? …….

55. Have you ever been exposed to radiation or asbestos? .....Yes No

56. Have you ever received treatment for emotional or mental problems? ……Yes No

SECTION F

(TO BE FILLED BY RESEARCHER ONLY)

Investigations and anthropometry

Anthropometry (pre-intervention) (post- intervention)

Weight…….

Height…….…….

Waist circumference……...

BMI…….……Muscle Mass……..

Visceral fat……Body Fats……

Investigation

Lipid profile……

Total cholesterol…….

LDL……..……

HDL……..……

Triglycerides……..…….

Sensory Analysis of *Borassus aethiopium* Bread.

Kindly tick your view on the set section in the box provided for options.

SECTION A

OLFACTION

	Pungent	Minty	Musky	Astringent
Sample 1				
Sample 2				
Sample 3				
Sample 4				
Sample 5				
Sample 6				
Sample 7				
Sample 8				

SECTION B

BASIC TASTE

Kindly tick your view on the set section in the box provided for options.

(SOUR)

	Like very much	Like extremely	Like Moderately	Neither like or Dislike	Dislike much	Dislike Very Much	Extremely Dislike
Sample 1							
Sample 2							

Sample 3							
Sample 4							
Sample 5							
Sample 6							
Sample 7							
Sample 8							

(SALT)

	Like very much	Like extremely	Like Moderately	Neither like or Dislike	Dislike much	Dislike Very Much	Extremely Dislike
Sample 1							
Sample 2							
Sample 3							
Sample 4							
Sample 5							
Sample 6							
Sample 7							
Sample 8							

(SWEET)

	Like very much	Like extremely	Like Moderately	Neither like or	Dislike much	Dislike Very	Extremely Dislike

				Dislike		Much	
Sample 1							
Sample 2							
Sample 3							
Sample 4							
Sample 5							
Sample 6							
Sample 7							
Sample 8							

(BITTER)

	Like very much	Like extremely	Like Moderately	Neither like or Dislike	Dislike much	Dislike Very Much	Extremely Dislike
Sample 1							
Sample 2							
Sample 3							
Sample 4							
Sample 5							
Sample 6							
Sample 7							
Sample 8							

(SAVORY)-UMANI

	Like very much	Like extremely	Like Moderately	Neither like or Dislike	Dislike much	Dislike Very Much	Extremely Dislike
Sample 1							
Sample 2							
Sample 3							
Sample 4							
Sample 5							
Sample 6							
Sample 7							
Sample 8							

SECTION C

Kindly tick your view on the set section in the box provided for options.

APPEARANCE (SIGHT)

	Very attractive	Moderately attractive	Not too Attractive	Not attractive at all
Sample 1				
Sample 2				
Sample 3				
Sample 4				
Sample 5				

Sample 6				
Sample 7				
Sample 8				

SECTION D

Kindly tick your view on the set section in the box provided for options.

(FIRMNESS)- ABILITY TO HOLD

	Very Good	Good	Fair	Poor
Sample 1				
Sample 2				
Sample 3				
Sample 4				
Sample 5				
Sample 6				
Sample 7				
Sample 8				

NOTE OF APPRECIATION

I DEEPLY EXPRESS MY WARM GRATITUDE FOR YOUR SUPPORT AND COUNCIL. THANK YOU.

Participant Information Leaflet and Consent Form

This leaflet must be given to all prospective participants to enable them know enough about the research before deciding to or not to participate

Title of Research:

Evaluating the impact of *Borassus aethiopum* "oman kube" on the lipid profile of individuals with cardiovascular disease.

Name(s) and affiliation(s) of researcher(s):

This study is being conducted by Dr. Charles Apprey, Prof. Dzogbefia and Marian Peprah all of the department of biochemistry, KNUST, Kumasi.

Background (Please explain simply and briefly what the study is about):

Non-communicable diseases particularly cardiovascular disease is on the increase and remain an area of high public health concern. Regardless of the increase in research in this area, and various approaches, prevalence is still high and lifestyle changes including diets rich in phytochemicals have been proven to be helpful. *Borassus aethiopum* a local plant has been noted for its high phytochemical content making it a good potential for lowering blood lipids. This research is aimed at evaluating the anti-lipidemic effect of borassus aethiopum on individuals with cardiovascular diseases.

Purpose(s) of research:

The purpose of this study is are as follows;

To look at how *Borassus aethiopum* can be used as composite flour in bread making and to evaluate

whether *Borassus aethiopum* can help in lowering blood lipid (cholesterol) in individuals with high lipid or cholesterol level

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

Anthropometric data and blood sample for lipid profile will be done. Each participant will receive weekly supply of the product (blinded bread). You will be required to eat the bread twice daily for four months. Blood sample for lipid profile will be done after the intervention. In total we expect to recruit 100 participants into this study, 50 each from 37 military and Legon hospitals

Risk(s):

Risks or discomforts associated with this Study are;

- pain involved in taking blood for sample
- Possible allergic reaction with *Borassus aethiopum*

The likely sign to expect in an allergic reaction with *Borassus aethiopum* are;

Hives

Itching (skin and throat)

Watery eyes

What to do when you have an allergic reaction

Stop taking the product immediately

Drink plenty of water

Move to the nearest hospital

Call the following numbers, **0553688853 or 0575646618 or 0243826275** as soon as possible

Submit all receipt of payment for medical services received to investigators for **FULL** reimbursement

Benefit(s):

- Daily supply of bread
- Benefit of being in the Study is to possible experience a low serum cholesterol and glucose.

Confidentiality:

All information collected in this study will be given code numbers to all names recorded. Only codes will be used for all electronic and non-electronic entries. The records of this study will be kept strictly confidential. Records will be kept by researcher alone in a locked file, and all electronic information will be secured using a password protected file No name or identifier will be used in any publication or reports from this study.

Voluntariness:

You are not under obligation to take part in this research. Taking part in this study is entirely voluntary (your free will)

Alternatives to participation:

If you choose not to participate, this will not affect your relationship with the investigators or your treatment in this hospital in any way.

Withdrawal from the research:

You have the right not to answer any single question, as well as to withdraw completely from the interview or refuse to take part in the study at any time and at any point during the process

Consequence of Withdrawal:

There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study, however, some of the information that may have been obtained from you before you chose to withdraw, may have been use and cannot be removed if analysis reports and publications have already been made.

Costs/Compensation:

No compensation will be made for your participation, however full payment will be made to participant due to cost incurred for allergic reactions.

Contacts:

If you have any question concerning this study, please do not hesitate to contact the following;

Dr Charles Apprey on 0243826275 and Marian Peprah on 0553688853 or 0576546618

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman

Committee on Human Research and Publication Ethics

Kumasi

Tel: 03220 63248 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained what this research is all about to …….. and have given him / her sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

Date: _____ Name……..

Statement of person giving consent:

I ----------have read the information on this study/research or have had it translated into a language I understand. I have also discussed fully with the interviewer all misunderstandings and enquiries to my satisfaction.

I understand that my participation is not compulsory (voluntary).

I know enough about the purpose, methods, risks and benefits of this research study and have decided to take part in it.

I understand that at any time I can freely stop being part of this study without having to explain myself to the researcher.

I have received a copy of this information leaflet and consent form to keep for myself.

Subject's Name : _____ **Date:**

Subject's Signature or thumbprint: _____ **Date:** _____

Investigator's Signature: _____ _____