# ASSESSMENT OF SOME HEALTH BENEFICIAL CONSTITUENTS OF EDIBLE PORTIONS OF FOUR UNDERUTILISED FRUITS

By

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## CERTIFICATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where the acknowledgement has been made in the text.

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## ABSTRACT

The surge in chronic diseases has necessitated the global call for increased consumption of diverse fruits and vegetables resulting in the need to study the potential health beneficial constituents of the underutilised. This research was aimed at assessing the dietary fibre fractions, proximate composition, antioxidant activity and phytochemical constituents of edible portions of four underutilised fruits viz., African mango (seeds and pulp), breadfruit (pulp), soursop (pulp) and sweetsop (pulp). Their total phenolic and ascorbic acid (vitamin C) contents were also determined. Samples for all determinations except proximate analysis were freeze-dried and stored: Some under refrigeration conditions for dietary fibre analysis, and the rest at - 20 $^{o}$C. Proximate and phytochemical analysis was done by standard methods. The antioxidant activity and phenolics were determined by spectroscopy using 2, 2-diphenyl-1-picrylhydrazyl radical scavenging and the Folin-Ciocalteu protocol, respectively. The ascorbic acid content was determined by titrimetry and an enzymatic-gravimetric method employed for the dietary fibre determinations. Proximate values (% mean) ranged from 2.63±0.11 to 6.71±0.07; 2.44±0.07 to 4.00±0.03 and 2.60±0.41 to 13.90±0.58 for protein, ash and fat respectively. Generally, tannins, triterpenoids, saponins, sterols, cardiac glycosides, flavanoids and coumarins were detected. Antioxidant activity of the samples ranged from 63% (breadfruit) to 78% (African mango pulp) and the highest phenolic content was recorded in African mango seeds. Total dietary fibre contents ranged from 11.50 (soursop) to 22.70 (African mango pulp) g/100g while ascorbic acid contents of the fruit mesocarps ranged from 20.32 (sweetsop) to 62.52 (soursop) mg/100g. There were non-significant correlations ($P < 0.05$) between the phenolics, ascorbic acid contents and antioxidant activities. The study suggests significant composition of health beneficial constituents - dietary fibre, vitamin C and presence of an array of phytochemicals - as well as fairly high antioxidant activities of the stated fruits. Thus, the need to exploit them in seeking optimum health of the populace.

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## CONTRIBUTION TO SCIENCE

- The study provides data on the total dietary fibre and its fractions (soluble and insoluble) of edible portions of *Annona muricata, Annona squamosa, Irvingia gabonensis and Artocarpus altilis*
- The findings of the study complement literature on the antioxidant activity and vitamin C content of fruit mesocarps of the four underutilised fruits

## PUBLICATIONS AND PAPER PRESENTATIONS FROM STUDY

- Boakye A. A., Oduro I. and Wireko-Manu F. D. B. (2013). Dietary fibre and proximate composition of selected Ghanaian underutilised fruits. Manuscript in preparation
- Boakye A. A., Oduro I. and Wireko-Manu F. D. B. (2013). Assessment of some health beneficial constituents of four underutilised tropical fruits. Manuscript in preparation
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## DEDICATION

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# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 Background

Fruits represent an abundant source of nutrients and non-nutritive bioactive compounds which are mostly associated with reduced risk of many non-communicable/chronic diseases (Wolfe *et al*., 2008). Despite the availability of a number of tropical fruits on the market, the varieties readily accessible to a greater proportion of consumers represent only a small fraction of the diverse and potentially beneficial species in nature.

A study by Williams and Haq (2000) demonstrated that most underutilised fruits have appreciable health and economic benefits. This has been confirmed by Padulosi *et al*. (2006) and Magdum (2006) with the added suggestion that most indigenous underutilised tree fruits have the capacity to contribute significantly to poverty alleviation and food security in low/ middle income nations.

The acclaimed health benefits of fruits in general, are mainly attributed to their dietary fibre composition and antioxidant activity which is largely characterised by the number and diversity of their phytochemical composition and to an extent, the vitamin C content. Vitamin C has other therapeutic effects including the prevention of anaemia and scurvy (Sochor *et al*., 2010; Delisle *et al*., 2011). Dietary fibre can be soluble or insoluble; the insoluble dietary fibre plays a major role in controlling postprandial serum glucose levels as well as in intestinal regulation to ultimately reduce the risk of colon cancer whereas its soluble counterpart is known to be essential in the prevention and treatment of obesity and diabetes (Champ *et al*., 2003). The additive and synergistic effects of inherent phytochemicals on the other hand, impart antioxidant and other peculiar toxicological/ pharmacological effects that are known to be responsible for the inverse associations between the intake of fruits and

chronic diseases such as coronary heart diseases and many cancers (Cartea *et al*., 2011; Liu, 2003). Thus, the more diverse the phytochemicals available in a diet, the better the health-beneficial potential derived.

Research into the prevention of the now global epidemic of NCDs recommends a minimum daily intake of five servings of fruits and vegetables to obviate for example, 31%, 19%, 19% and 12% of ischemic heart disease, ischemic stroke, gastric cancer and lung cancer respectively (Hall *et al*., 2009; Anonymous, 2010). However, fruit and vegetable consumption as reported by WHO/FAO (2004) showed very low consumption trends among low/middle income nations with sub-Saharan Africa recording an annual consumption between 27 – 114 kg/ capita compared to the recommended per capita intake of 146 Kg . Of this abysmal performance, vegetable consumption was nonetheless observed to be almost universal and a little bit more appreciable than that of fruits which was less common in diets and its prevalence even more varied among the studied nations (WHO, 2005). The trend is similar in Ghana where inspite of a relatively better reported prevalence of fruit and vegetable consumption - 62% for women and 63.4% for men as compared to that of other African countries, the reported annual per capita intake of fruits, 23.5 kg, is still far below the recommended (Hall *et al*., 2009). These rates, especially in the urban communities, are highly dependent on the seasonality and relative abundance of some few common fruits (WHO, 2005).

In seeking workable solutions to curb the growing phenomenon of low consumption of unrefined plant-based diets and rising chronic diseases, Padulosi *et al*. (2006), recommended plant biodiversity with emphasis on indigenous but underutilised species to significantly contribute to the realisation of domestic food security as well as help curtail the double burden of malnutrition especially in low/middle income nations. Similar calls have been made for the numerous lesser known Ghanaian fruits including *Annona muricata, Annona*

*squamosa*, *Diospyros mespiliformis*, *Gardenia erubescens*,, *Irvingia gabonensis*, *Artocarpus altilis* and *Detarium microcarpum* (Abebrese *et al*., 2007; Aboagye *et al*., 2006).

An appreciation of this concept in the recent past, has led to a paradigm shift of researchers into exploring the optimal use of underutilised tree fruits with emphasis on their non-nutritive but otherwise health-beneficial constituents, dietary fibre and phytochemicals, vis - a - vis their established role in combating the now – universal menace of NCDs (Hall *et al.,* 2009). To wit, a number of low/middle income nations have explored the value of most of their indigenous but underutilised fruits in order to aid productive inputs in encouraging their increased consumption, value addition, probable use in food formulations and fortifications, and their subsequent commercial production in ensuring food security (Marinov *et al*., 2005).

Gomes de Melo *et al*. (2010) reported an array of phytochemicals including alkaloids, tannins, saponins and sterols with a corresponding high antioxidant activity for inedible morphological parts of Brazilian varieties of *Annona muricata* (soursop) and Arthur *et al*. (2012) reported the efficacy of leaf extracts of some Ghanaian varieties against jaundice. Adewole and Caxton-Martins (2006) also proposed the use of the fruit pulp as lactagogue and as cure for diarrhoea and dysentery in Nigeria. Kolar *et al*. (2011) reported the health benefits of some Indian varieties of *Artocarpus altilis* (breadfruit) pulp while Ragone, (2011) suggested its potential as replacer for staple crops to combat hunger and poverty in the Caribbean. In addition, the leaves of *Annona squamosa* (sweetsop) have been determined to have health beneficial constituents that have inverse relations with obesity and diabetes (Biva *et al*., 2009; Shirwaikar *et al*., 2004).

## 1.2 Problem statement

There is dearth of information on the health beneficial constituents of the edible portions of indigenous underutilised fruits in Ghana to make any substantial claim for their optimal use (WHO/FAO, 2004; Abebrese *et al*., 2007).

Endeavours in research works to enhance the commercial production and subsequent value addition of fruits, have been skewed towards the hackneyed ones leaving no room for variety for consumers. This challenge is exacerbated by recent policy that focuses on the common few fruits such as pineapple, citrus and pawpaw in a bid to diversify the nation's agricultural export base to ensure food security through non- traditional export commodities (Aboagye *et al*., 2006; FASDEP II, 2007).

## 1.3 Justification

Investigating key parameters for assessing the health potential of the underutilized fruits will complement literature in making known the potential of the Ghanaian underutilized species as well as provide a stepping-stone for further research works in other uses of the fruits.

Currently, consumers choose diets based on the associated nutritional and health benefits instead of taste (Katan and De Roos, 2004) and it is postulated that the published information obtained on the not-so-known fruits will offer diversity to the health-conscious consumer. This will curtail the challenges of monotony associated with fruit consumption and poor availability associated with seasonal variations. The knowledge obtained from the study, will also aid policy-makers in making informed decisions in the agricultural sector aimed at broadening the nation's food security basket.

## 1.4 Objectives

The overall aim of this research is to assess some health beneficial constituents of four underutilised fruits.

Specifically, the study seeks to:

1. Determine the dietary fibre fractions and proximate composition of the edible portions of *Annona muricata, Annona squamosa, Irvingia gabonensis* and *Artocarpus altilis*
2. Assess their phytochemical composition and determine their antioxidant activity

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Fruit consumption trends

It is said that, "The most political thing one does every day is in choosing what to eat" (Jeanette Winterson). According to Morgan (2009), one most unfortunate trend in diets currently, is the paradigm shift from whole plant foods especially those rich in fruits and vegetables to highly refined diets in low/middle income nations. This dietary pattern used to be the woes of the West with associated chronic diseases viz., hypertension, gastrointestinal disorders, cardiovascular diseases, diabetes and many cancers (FAO/WHO, 2004; Hall *et al*., 2009). The consumption trend, as is now being witnessed in developing countries have led to what is popularly referred to as "the double burden of malnutrition" that is, a section of the populace is rich, westernised and over-nourished (more animal sources, fewer plants and highly refined foods that is, having poor diets) with associated non-communicable (rich-man's) diseases whereas the rest are poor, provincial and undernourished (with associated poor man's diseases). This phenomenon is the current canker eating away developing countries (Anonymous, 2006).

A survey by Hall *et al*. (2009) showed low prevalence of fruit and vegetable consumption in all developing countries studied (including 18 from Africa) with prevalence of consumption ranging from 63.4% and 62% in Ghana to 0.8% and 0.7% in Pakistan for men and women respectively. Inspite of the relatively high prevalence observed in Ghana, obtained data showed neither Ghana nor any of the studied developing countries attained the USDA (2005) recommended per capita minimum quota of 146 kg/day of consumption of fruits and vegetables. The researchers also postulated that about 3 million deaths and 26 million disability adjusted life years was attributed to inadequate consumption of fruits and

vegetables – a trend that could be easily addressed with at least five servings of fruits and vegetables daily. In Africa, the prevalence of vegetable consumption is better than that for fruits as vegetables mostly form a major part of items in native dishes (Hall *et al*., 2009; FAO/WHO, 2004) indicating a lower prevalence of fruit consumption compared to that of vegetables. Thus, the need for much emphasis to be placed on fruits in the quest for optimal health benefits from plant-based diets. Moreover, fruits are usually eaten whole and raw and therefore do not require much processing – a major factor in the loss of inherent beneficial constituents of most plant foods (Tosun and Yücecan, 2007; Kadar, 2002).

Suggested solutions aimed at increasing fruit consumption in developing nations include, creating awareness of their health potential as well as incorporating variety preferably, through the use of health beneficial underutilised species, into the existing fruit market to rejuvenate their consumption (Padulosi *et al*., 2006; FAO/WHO, 2004). However, the challenge here is the lack of information especially on the beneficial constituents of the indigenous underutilised species (Hawtin, 2007).

### 2.2 Underutilised plant species (UPS)

The focus of 21$^{st}$ century researchers is on developing and improving the use of plant species hitherto ignored by science and technology, preservation methods, marketing systems and management efforts (Padulosi *et al*., 2006; Horna *et al*., 2007). According to Padulosi *et al*. (2012) this global trend spearheaded by organisations including the Bioversity International, Global Facilitation Unit on Underutilised Species (GFU), Food and Agricultural Organisation (FAO) and Global Forum on Agricultural Research (GFAR), has been necessitated by the reliance of humans over the generations on comparatively fewer food crops. Of the known hundred thousands of edible plant species worldwide, FAO (2009) reports only 20 is presently grown to provide for about 90% of the world's plant-based energy, a trend that has

resulted in the shrinking of the food basket to the detriment of low/middle income earners whose survival and nutrition depends on crop diversity (Padulosi *et al*., 2006).

Paramount among these ignored species now with greater scientific focus in light of growing diet-related diseases are fruits and vegetables (WHO/FAO, 2004). Some tree fruits identified as being underutilised in Ghana include, *Diospyros mespiliformis, Irvingia gabonensis, Detarium microcarpum, Limonia acidissima, Aegle marmelos, Annona muricata, Annona squamosa, Artocarpus altilis, Tamarindus indica* and *Gardenia erubescens* (Hawtin, 2007; Abebrese *et al*., 2007; Jaenicke and Höschle-Zeledon, 2006). Most of such fruits are indigenous to other low/ middle income nations (Padulosi *et al*., 2006).

These are hypothesised to have health potential comparable to the overused ones and also have the capacity to significantly contribute to food security in low/ middle income nations as well as help poorer nations reach their millennium development goals (MDGs). Success stories such as the use of the leaves of *Annona muricata* (soursop) and *Artocarpus altilis* (breadfruit) for the treatment of hypertension in Trinidad and Tobago (Lans, 2006) as well as the successful commercialisation of juice from *Annona Muricata*, *Limonia acidissima* (wood apple) and *Aegle marmelos* (bael fruit/ stone apple) by a rural Sri Lankan peasant who earned USD 500 monthly and employed ten local barely two years after establishment, corroborate this notion (Jaenicke and Höschle-Zeledon, 2006).

Identified primary constraints hindering the promotion of the myriad of underutilised plant species (UPS) in low/ middle countries and especially in sub-Saharan Africa include lack of awareness, education and knowledge on their health and economic potential; inadequate policies and marketing systems for their commercialisation as well as unavailable technologies for production, post-harvest management, value addition and packaging of the UPS (Padulosi *et al*., 2012; 2006). Of the number of strategies proposed for the ultimate

utilisation of UPS, research and development on their individual potentials is top priority (Hawtin, 2007).

These plant species usually generate controversy over the appropriate terminology to call them or the criteria to use in classifying a species as one. This is so because the terms commonly used such as, underutilised/underused, neglected, orphan, minor, traditional, novel, promising, lost, niche and new crops (Gruere *et al*., 2006) do not give any information on their geographical, economic and social implications with some given names apparently misconstruing the potential of the species. Agreeably, it is imperative for any chosen name to be defined in the context in which it is being used (Padulosi *et al*., 2006).

In accordance with Jaenicke and Höschle-Zeledon (2006), underutilised crops in this study refer to those species in a given locality with underexploited potential in relation to their health, economic and environmental benefits as well as their contribution to national food security, income generation of rural folks and alleviation of extreme poverty and hunger.

### 2.2.1 Demand for Underutilised Fruits (UF)

The therapeutic prospects of tropical tree fruits resulting from their inherent bioactive compounds has over the years gained recognition both domestically and abroad. Thus, various morphological parts of a number of them have been researched into for their possible pharmacological/ toxicological use plus their use in food formulations and or fortifications (Ruffino *et al*., 2010).

Currently, the global epidemic of chronic diseases coupled with the increasing challenge of the double burden of malnutrition in low/middle income nations, has revamped the call for consumption of more fruits and vegetables because of their being a cheaper and effective source for curtailing this challenge (FAO/WHO, 2004; Hall *et al*., 2009). The trade globalisation remains an important factor in determining which fruits are researched into and

subsequently marketed. According to Katan and De Roos (2004), consumers now rate and patronise food items based on associated health benefits rather than taste: a health-conscious attitude which is gradually spearheading the politics of trade globalisation.

This growing outlook of consumers has provided a greater opportunity for many lesser known species to be researched into vis – á – vis their contribution to curbing the growing menace of non-communicable diseases and consequently their demand in both international and local markets (Rawat *et al*., 2010). Thus, the prospective long-term contributions of underutilised tree fruits in ensuring national food security by enlarging the food basket of impoverished nations as well as boosting their economies by alleviating rural poverty cannot be overstated. This vision is steadily being made a reality with a number of indigenous but underutilised fruits in some low/ middle income nations but there is still more to be done (Vera de Rosso, 2013; Ragone, 2011; SCUC, 2006; FAO/WHO, 2004).

### 2.2.1a *Annona species*

#### *Origin and distribution*

The *Annona* species are from the family Annonaceae and as the name suggests, varieties of this genus generally have annual fruit production (SCUC, 2006). About 119 species have been identified in the genus and some common ones with edible fruits include; *muricata* (soursop), *squamosa* (sweetsop), *senegalensis* (wild soursop), *reticulata* (custard apple), *cherimola* (cherimoya) and the hybrid, *atemoya* (cross between *cherimoya* and *squamosa*) (Orwa *et al*., 2009). The genus has a wide distribution spanning both tropical and subtropical regions including America, Africa and mid-Asia with *A. muricata* and *A. squamosa* being the most widely distributed species. *A. cherimoya*, *A. muricata* and *A. squamosa* are those in highest consumer demand with a corresponding importance in local, national and regional trade though scarcely known in international trade. Inspite of their wide distribution, a review

by Pinto *et al*. (2005) on research works and utilisation of various morphological parts of the above-mentioned species show the fruits are underutilised in most indigenous areas.

***Annona muricata* L. (Soursop)**

The *Annona muricata L.* commonly referred to as soursop, graviola, sir sak and corrosol is locally known as 'alunguntugui', 'apre' and 'evo' in the Ga, Twi and Ewe dialects, respectively (Personal communication). A number of countries such as India, Columbia, Costa Rica and Angola undertake commercial production of the species with Venezuela, Mexico and Brazil being the major world producers. Despite a dearth of information on the production levels of the fruits in various regions, Mexico has been suggested to be the leading world producer with a per capita production of 34,900 MT in 1997 (Pinto *et al*., 2005). In these countries, the *Annona muricata* tree, postulated to have higher industrial value than most species from the same genus, has a number of uses; fruit pulp eaten as food, stem for timber and most parts including leaves, stem and fruit seeds used as medicine (Orwa *et al*., 2009).

Soursop has varying fruiting seasons for different varieties with Hawaiian varieties having three seasons; January – April (for early crop), June – August with peak in July (for mid-season) and late October – November (for late crops) (Dembitsky *et al*., 2011). The fruit is highly perishable (stays wholesome for 2–5 days when harvested at commercial maturity) due to its high moisture content - a viable industrial potential - necessitating the need for value addition to extend its shelf-life (Lima and Alves, 2011).

In Ghana it is usually found in the wild in most areas except the three Northern regions and is sparsely cultivated in various communities basically for its fruits and ornamental purposes (Arthur *et al*., 2012). However, there are no records on production levels of the fruit. One major fruiting season, late June through to August with peak in July, has been identified

(Personal communication). Moreover, its use is limited to consumption of the fruit pulp as snacks and the tree for ornamental purposes in localities where they are dominant. Thus, the need for further studies in order to expand its utilisation base.

***Health potential of soursop***

A review by Rajeswari *et al*. (2012) showed a number of works done on the phytochemical and pharmacological properties of the inedible portions - seeds, leaves and roots of the soursop tree but with little done on the fruit pulp. The researchers suggested the identified chemicals viz., acetogenins such as annomuracins (A and B), annopentocin and annonacins to be responsible for the potency of the morphological parts as cure against ailments including some cancers.

In addition, the leaves and seeds of the soursop tree have been identified by Usunomena (2012) and Gomes de Melo *et al*. (2010) as having appreciable antioxidant activity (AOA) supporting their folkloric medicinal use against risk of non-communicable diseases such as hypertension and diabetes. A number of other studies report an array of phytochemicals including terpenes, tannins, glycosides, flavanoids and alkaloids in extracts of the leaves, bark and seeds (Pinto *et al*., 2005) with epidemiological studies confirming the antioxidant, hypoglycaemic and hypolipidemic properties of the leaf extract in experimental rats (Adewole and Ojewole., 2008). In Ghana, Arthur *et al*. (2012), substantiated the folkloric use of leaf extracts of indigenous varieties in the treatment of jaundice by studying its activity against temporary jaundiced mice after an earlier basic phytochemical screen (Arthur *et al*., 2011) that detected the presence of tannins, glycosides, saponins and flavanoids. All these determinations further corroborate the folkloric therapeutic use of various inedible morphological parts of the soursop.

A review by Dembitsky *et al*. (2011) reports the fruit mesocarp of soursop has an appreciable nutritional value with considerable amounts of carbohydrates, proteins and vitamin C in the fruit pulp of some American and Caribbean varieties. This has been corroborated by Enweani *et al*. (2004) who showed the fruit mesocarp has substantial mineral contents postulated to effectively produce the electrolytes needed in oral rehydration therapies. The fruit pulp is also postulated to have essential oils and studied varieties of the exotic fruit pulp from Cameroon yielded appreciable amounts of aliphatic acids and their esters (Jirovetz *et al*., 1998). However, there are varying reports of the crude fat content of the species from different localities (Folorunso and Modupe, 2007; Abbo *et al*., 2006; Enweani *et al*., 2004) – a phenomenon that may be occurring from varietal differences. The fresh pulp is used in folkloric medicine for treating constipation and some cardiac disorders (Dembitsky *et al*., 2011).

However, there is dearth of literature on the AOA of varieties of the fruit pulp (Usunomena, 2012) and although a number of studies report the crude fibre content, rarely do they analyse its nutritional value in terms of the dietary fibre composition. These parameters are yardsticks for measuring the potential of the fruit in imparting some of these traditionally acclaimed health benefits. This is especially so in Ghana where inspite of a growing popularity of the fruits of soursop especially, much of the research is on the leaves and seeds neglecting the fruit mesocarp (Personal communication; Arthur *et al*., 2012).

It is worth mentioning that toxic secondary metabolites such as the neurotoxin, swainsonine, have been detected (in very minute amounts) in the fruit mesocarp of some Brazilian varieties by Mohanty *et al*. (2008) raising concerns over their safety as food. However, inspite of the absence of clinical trials to validate this concern, reports show the detected amount (0.0004%) is far below the threshold for toxic effects in ruminants (Mohanty *et al*., 2008). The identified health benefits associated with the fruit consumption (some afore-mentioned)

in addition to its folkloric use over decades without any identifiable side effects override this food safety apprehension.

***Annona squamosa***

This specie is popular for its unique sweet taste and has common names depicting this property: sweet apple, sugar apple, sweetsop, custard apple and cinnamon apple (Pandey and Barve, 2011). It is the most widely distributed of the genus in the tropics, found in coastal areas of tropical America and cultivated in most African nations as well as in Malaysia, Sri-Lanka, Philippines, Australia, India, Polynesia and the Malay Archipelago. Although there is scant data on the production levels of sweetsop, Philippines which still has the fruit as a backyard fruit with mainly domestic consumption, is considered a major producer with a reported production of 6,262 MT in 1978. Moreover, available information suggests the potential of expanding the market for sweetsop in many countries in which it is indigenous (Pinto *et al*., 2005). Generally, the *A. squamosa* tree has one harvesting season from July – September but agronomic interventions yield another harvesting period from October to March (Pinto *et al*., 2005). The fruit pulp is usually eaten fresh or frozen and also processed into jams and nectar but so far has a lower reported industrial use compared to *A. muricata* (Pinto *et al*., 2005) and known to be underutilised in many indigenous areas (SCUC, 2006).

In Ghana, the Ashantis call the fruit 'sweet apre' (for sweet apple in English) and it has a peak harvest in August. It is commonly found in the wild in the central, Volta and Ashanti regions. There are no local records on commercial production levels of the fruit although the fruit is popular among the youth in communities where it is endemic. Largely due to surrounding myths and lack of scientific evidence to prove otherwise, the fruit is limited to being eaten as snacks by mostly children in communities where they are dominant in Ghana – indicating the need for further analysis to determine their economic and health potential.

***Health potential of sweetsop***

Patel and Kumar (2008) and Ramesh *et al*. (2011) reported folkloric medicinal use of all parts of the tree; roots ingested to alleviate depression and spinal disease, fruits used as blood tonic, oil and resin from seeds used as detergent and leaves used as poultice on boils and ulcers. Pandey and Barvey (2011) also reported high AOA of *A. squamosa* leaves in addition to epidemiological studies (Shirwaikar *et al*., 2004) that confirmed their antidiabetic properties as indicated in folkloric medicinal use. To further confirm the antioxidant potential of the leaves, Biva *et al*. (2009) reported the presence of saponins and tannins in studied leaf extracts. A review by Pinto *et al*. (2005) showed the presence of bioactive compounds in the roots and bark of the sweetsop and these compounds are known to impart antibacterial, anticancer and anti-depressing properties. The researchers found the seed to be effective as an insecticide against lice and bed bugs as well as being employed for human abortions. In summary, the reviewers suggested all inedible morphological parts of the plant as having antibacterial properties as well as being used to treat dysentery, diarrhoea and pneumonia.

Much research has not been done on the fruit mesocarp and Pinto *et al*. (2005) proposed very little nutritional importance for the fruit mesocarp. The reviewers reported the pulp of sweetsop to have low protein and fibre content but an appreciable vitamin C composition. However, a review by Dembitsky *et al*. (2011) on other varieties, suggested the presence of health beneficial constituents such as alkaloids and cardiac glycosides in addition to the detection of an array of minerals in the fruit mesocarp. Nwokocha and Williams (2009) also reported an appreciable fat and carbohydrate composition in the fruit mesocarp debunking the assertion by Pinto *et al*. (2005) and also indicating the need for more work to be done on the fruit pulp to ascertain their health potential.

### 2.2.1b *Irvingia gabonensis*

*Irvingia gabonensis* is postulated to be from the family Simarubaceae (Ayivor *et al*., 2011) but this is challenged by some taxonomists because 'bitter principles', a major characteristic of the family, is absent in *I. Gabonensis*. It is currently accepted to belong to a family of its own, *Irvingia* ceae (Ainge and Brown, 2001). *I. gabonensis* bears mango-like fruits and is thus commonly referred to as the African bush mango (Matos *et al*., 2009). The tree fruit is widely distributed in the tropical and subtropical forests of West and Central Africa (Ayivor *et al*., 2011) spanning Liberia and DR Congo. The fruit is dominant in Cameroun, Gabon, Nigeria and Côte d'Ivoire and there is a high market demand especially for the kernels in these regions. Of these, Cameroun is considered a major producer and exporter of the fruit. A production of 1112 kg/ grower or collector was reported in 1999 alone for the humid lowland communities in the country (Ainge and Brown, 2001). In Nigeria, its seeds, called "ogbono", form the main ingredient for the popular 'ogbono' soup (Ekpo *et al*., 2007) and the country is both a source and destination of trade for the fruit. It is known as "borborou" and "bulukutu" in Côte d'Ivoire and Cameroon, respectively (Ainge and Brown, 2001). In Ghana, the fruit is found in the wild and it is dominant in the southern parts (Ayivor *et al*., 2011) especially in the Volta region where it is locally known as 'anyikele' (Personal communication). However, documentation on their production levels is not readily available although the indigenes export the fruit to neighbouring countries.

The *Irvingia gabonensis*, hereafter referred to as African mango, is the eating specie of the genus and is often confused with *Irvingia wombolu* which has an inedible and more fibrous pulp but preferred seeds/ cotyledons (Ekpo *et al*., 2007). There are varietal differences in the African mango with variations occurring in fruit production, sweetness, colour, shape and nutritional quality. The specie also varies in ease of seed cracking and flowering as well as in its fruiting phenology (Ladipo *et al*., 1998).

The tree fruit is classified as an indigenous underutilised tropical wild fruit (Aboagye *et al*., 2007) that is an important tree fruit for domestication (Ayivor *et al*., 2011; Ladipo *et al*., 1998) and for curtailing the double burden of malnutrition in Africa (Anonymous, 2006). The fruits are usually harvested mature but unripe and then stored to ripen to reduce insect attack (on the tree) and subsequent spoilage (Ainge and Brown, 2001).

***Health potential of African mango fruit***

Etebu (2012) suggests the potential of the edible fruit pulp is limited unlike its seed which is the most desirable portion in diets of indigenes. Thus, studies on the fruit are usually focussed on the seeds. Epidemiological studies support the potential use of the seeds in the treatment of diabetes and obesity (Ngondi *et al*., 2005) and Etebu (2012) reported the presence of health beneficial phytochemicals such as alkaloids, saponins, glycosides and tannins. In line with the diverse phytochemicals reported as being present in African mango seeds, Agbor *et al*. (2005) established a relatively high phenolic content with correspondingly high total AOA but low vitamin C content. The seeds have also been postulated to be high in fibre by Ngondi *et al*. (2005) although data on dietary fibre contents by approved methods is scarce.

On the other hand, limited data on the fruit mesocarp reports the presence of major phytochemicals such as tannins, alkaloids, glycosides and flavanoids (Etebu, 2012). Ainge and Brown (2001) indicated the potential of the pulp in the juice, jam and jelly industry while Okiei *et al*. (2009) quantified its vitamin C content. Little is known about the antioxidant activity and dietary fibre composition of the pulp to substantiate any claims on their associated health potential. Thus, more studies have to be done to optimally tap the potential health and economic benefits of the fruit pulp (Etebu, 2012; Ainge and Brown, 2001).

### 2.2.1c *Artocarpus altilis*

*Artocarpus altilis* from the family Moraceae has other scientific (though not officially accepted) names such as, *A. camasi, A. mariannensis, A. communis* and *A. incisa* (Ragone, 2006). It is commonly referred to as breadfruit in English but has an array of names especially in the Pacific Islands where it is dominant (Appiah *et al*., 2011). In Ghana it is fondly called 'bayerɛ' (Twi for yam) because of the significant role it played in hunger alleviation in a national famine in 1983 (Personal communication).

The fruit has pantropical distribution spanning the Carribean to Central and South America, through Africa, India, Southeast Asia, Northern Australia to South Florida (Ragone, 2011). With an estimated yield of 4 tonnes/ hectare to 50 tonnes/ hectare (Jones *et al*., 2011), a review by Ragone (2011) showed high per capita production of the fruit in the Carribean and Pacific with the Fiji Islands alone recording a total export of 12 MT of fruits in 2005. Data on production in Africa however, is scarce (Ragone, 2011) and there is no official documentation for that of Ghana.

Researchers cite one major fruiting season for most varieties with some having a second minor season usually 3-4 months after the first (Ragone, 2011). The trend is same in Ghana where the fruit, predominantly found in the wild in most areas except the three Northern regions, has a minor fruiting season from January – March and a major one from July – September with peak in August (Personal communication). The fruits of this specie vary in shape, size and surface texture and the tree has been reported to flower and fruit from 3 - 6 years (for vegetative propagation; usually employed for seedless varieties) and 6 - 10 years by seed cultivation (Ragone, 2006). There is reported folkloric medicinal use for almost all morphological parts of the tree in addition to parts being used as fodder, timber, gum and textiles. The latex from the fruit is also used as adhesives (Orwa *et al*., 2009). The fruit, inspite of its wide distribution, high yield, potential for low input and sustainable production

systems, remains underutilised outside the Pacific (Ragone, 2006; Appiah *et al*., 2011). This challenge is due in part to its wide varietal differences which makes it difficult to extrapolate results from one indigenous area to another (Ragone, 2011; Ragone, 2006).

***Health potential of breadfruit***

A review by Jones *et al*. (2011) identified over 130 chemical compounds (mostly phenylpropanoids such as flavanoids and flavones) in various morphological parts of some varieties of breadfruit including sterols in the fruit pulp. Kamal *et al*. (2012) also detected coumarins, tannins, glycosides and phenols in the twigs of the plant supporting their folkloric medicinal use.

Available data on the fruit pulp suggests breadfruit to be nutritious in terms of its mineral, protein, crude fibre and carbohydrate content (Adekunle and Oyerinde, 2004). As a result of the high carbohydrate content, Ragone (2011) showed its potential as a good replacer of flour in the bakery industry as well as the possibility of it being used to replace staple crops (especially when out of season) to ensure food security and adequate supply of nutrients in developing nations.

However, Jones *et al*. (2011) showed significant variations in the chemical composition of breadfruit in the various reports reviewed. Ragone (2006; 2011) attributed the variation in data to wide varietal differences (even within the same locality) in the specie thereby establishing the need for an in-depth analysis of the specie in each place of origin to adequately assess its associated health and economic potential. The review by Jones *et al*. (2011) further indicated limited literature (especially on the fruit pulp) on other health beneficial constituents to convincingly support their many traditional health claims.

### 2.3 Health beneficial constituents of fruits

From the above discourse, it is evident that in addition to their nutritional value, assessment of the total antioxidant activity (Pisoschi *et al*., 2009), inherent antioxidant vitamins (Russo, 2010; Lee and Kadar, 2000), phytochemicals (Liu, 2003) and the dietary fibre composition (Anderson *et al*., 2009; FAO/ WHO, 2004) of fruits are essential parameters for determining their health potential.

#### 2.3.1 Antioxidant activity (AOA)

Antioxidants are substances that can considerably hinder or prevent oxidation of oxidisable substrates such as nucleic acids and lipids when they are present in concentrations comparatively lower than that of the substrate in which they are found. Thus, antioxidants avert oxidation chain reactions of biomolecules (Wang, 2000). According to Brewer (2011), most antioxidants achieve this by scavenging free radicals (these are said to be the most effective antioxidants) while some others do so by chelating metal ions and/ or quenching oxygen radicals in the system (Perron and Brumaghim, 2009; Anonymous, 2004). A free radical is a stable molecule with an unpaired electron that is highly reactive and capable of initiating degenerative chain reactions in biological systems (Wolfe *et al*., 2008). Antioxidants on the other hand, may be enzymes (predominantly superoxide dismutase, catalase and peroxidase) or non-enzymatic compounds such as ascorbic acid and phytochemicals including flavanoids, tannins and phenolic acids (Sochor *et al*., 2010).

Thus, the total antioxidant activity of a molecule is defined by Sochor *et al*. (2010) as "an ability of the compound (or mixture of compounds) to inhibit oxidation reactions of various biomolecules (such as prevent the peroxidation of lipids) in a system". In biological systems, in addition to scavenging free radicals, antioxidants may also act as antimicrobial and antiviral agents as well as immuno-stimulants – showing an array of health importance

(Murillo *et al*., 2012; Rawat *et al*., 2010). Although antioxidants are not a panacea for all ailments, the importance of naturally occurring non-enzymatic dietary antioxidants in reducing the risk of fatal diseases such as coronary heart diseases and some cancers has been established (Hall *et al*., 2009; USDA, 2005). For most foods therefore, the free radical scavenging properties of their inherent antioxidants is used as an indicator of their potential health benefits (Vera de Rosso, 2013; Pisoschi *et al*., 2009).

***Methods for AOA determination***

Antioxidants basically inhibit excess free radicals by single electron and/ or hydrogen atom transfer (Ozgen *et al*., 2006) and they can therefore be determined by measuring either the direct reactions between the antioxidant under study and their corresponding scavenging ability or the reactions of the antioxidant with trace metals (Sochor *et al*., 2010). Thus, a number of protocols have been proposed for their assessment viz., 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEAC), Oxygen radical absorbance capacity (ORAC), N,N-dimethyl-1,4-diaminobenzene (DMPD), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and free radicals kit (Sochor *et al*., 2010).

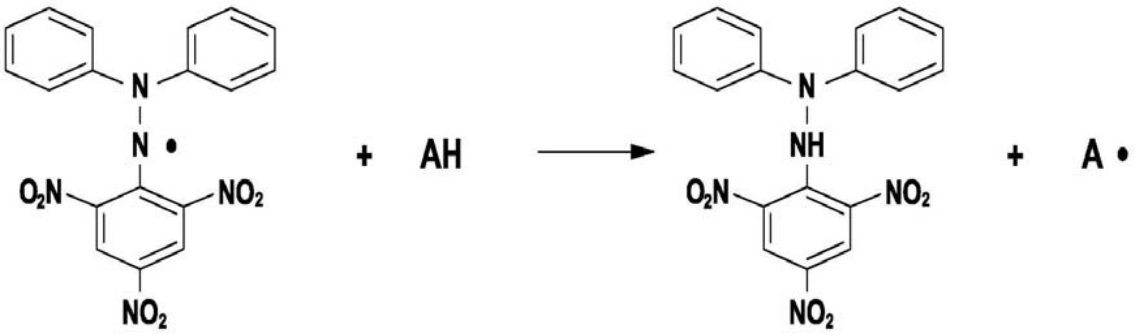
It is worth mentioning that all the methods give only an approximation of the actual AOA of natural (biological) substrates (Ozgen *et al*., 2006) and this is partly attributed to the fact that the efficacy of each of the methods is influenced by the chosen reaction time, extraction method(s) employed, the complexities of the kinetics of the reaction and the inherent potential for existing phenolic constituents to interact or polymerise. Thus, to simplify their evaluation, the antioxidant activities of foods are presented by referencing them to common standards such as (S) - (-) – 6 – hydroxyl - 2, 5, 7, 8 – tetramethylchroman - 2 - carboxylic

acid (commonly called Trolox) and gallic acid or expressed as a percentage of the reagent used (Prakash *et al*., n.d).

DPPH, FRAP and ABTS have been established as appropriate methods for AOA determination in fruits (Ozgen *et al*., 2006). Of these, the use of DPPH has been reported by Prakash *et al*. (n.d) to be particularly desirable for substrates with inherent diverse antioxidants such as fruits. The researchers suggested it is not hindered in activity by the physical state (whether solid or liquid) of the substrate under study as well as its ability to efficiently measure the total AOA of the substrate without necessarily being specific to any particular antioxidant present – a plus for total AOA determinations.

***DPPH***

The method has been widely used in assessing the AOA of foods and is fairly simple, cheap and quick to use (Pisoschi *et al*., 2009). DPPH measures the AOA of a compound by single electron transfer through a mechanism as shown in Fig 2.1.




**Stable radical (DPPH)** **Reduced (DPPH-H)**

Source: (Pisoschi *et al*. 2009)

**Fig 2.1: Mechanism for DPPH free radical scavenging**

The DPPH free radical, unlike other free radicals does not dimerise and is stable due to the delocalisation of the free electron. It is purple with strong maximum ultraviolet-visible spectrum absorption at around 517 nm. In the presence of an antioxidant (AH - a hydrogen donor), the DPPH is reduced to DPPH-H; it is thus decolourised (to yellow) with a corresponding decrease in its absorption strength. The decolourisation is stoichiometric with a direct correlation to the number of electrons captured at each point in time (Pisoschi *et al*., 2009; Prakash *et al*., n.d).

### 2.3.2 Antioxidant vitamins

Antioxidant vitamins refer to naturally-occurring vitamins and in this context, those in plant foods, with appreciable AOA. They include predominantly, vitamins C and E but not beta-carotene which is only a pro-vitamin for vitamin A, which by itself has no AOA (Russo, 2010).

Vitamin E is the common name for lipophilic compounds of two families, tocopherols and tocotrienols. Of these, Vera de Rosso (2013) reported the most potent and commonly found in foods to be α-tocopherol which is acclaimed for its AOA. Major sources of vitamin E include nuts, oils, seeds and wheat but very few fruits (Russo, 2010). Thus, it is not generally sought for in assessing the health beneficial constituents of most fruits.

Vitamin C, otherwise referred to as L- ascorbic acid (which is the active form of vitamin C existing as a hydrogen donor), is water-soluble and known to have inverse relations between its intake and the occurrence of scurvy. It is also noted for its contribution to the prevention of anaemia, fatigue, maintenance of the skin and teeth and treatment of wounds (Phillips *et al*., 2010). Vera de Rosso (2013) reported of its significant contribution to the total AOA of most plant foods. Lee and Kadar (2000) suggested vitamin C to be the most essential vitamin

available in fruits and vegetables thereby establishing it as a determining factor in assessing the nutritional and health potential of fruits.

***Mechanism of AOA activity of vitamin C***

Ascorbate/ L-ascorbic acid in biological systems acts in two main ways: directly and indirectly. In the indirect pathway, it stimulates inherent vitamin E to act as an antioxidant on the lipophilic membranes of the system. In the direct process however, ascorbate acts directly on membranes to prevent lipid oxidation by donating hydrogen atoms to the excess free radicals which usually occur as reactive oxygen and nitrogen species (ROS and RNS) in the system (Vera de Rosso, 2013). By so doing, it inhibits the degenerative activities of ROS and RNS. L-ascorbic acid is in the process, reversibly oxidised to L-dehydroascorbic radical which also has AOA. Upon further reaction, the L-dehydroascorbic radical is irreversibly changed to the diketogulonic acid which has no biological activity (Okiei *et al*., 2009).

Vitamin C is highly unstable, a property attributed to its strong reducing potential. As such, it is easily deactivated by most oxidising agents and it is upon this property that methods have been proposed for its quantification in foods. One such rapid and efficient method is the approved titrimetry through the use of redox reactions. This protocol employs the use of a stabilizer and a titrant. Commonly used titrants are the 2:6 dichlorophenol Indophenol (blue dye) and N-bromosuccinimide.

There are reported limitations with the use of the blue dye (Okiei *et al*., 2009) and one major setback is that it is not easy to detect the end point when analysing highly coloured samples. Its intense blue colour (used as a marker for determining the reaction end point) is also easily reduced making their determined end points unreliable. Moreover, the purity of the compound is not readily reproduced, a factor essential for the reproducibility of results. Thus

for most parts, N-bromosuccinimide is used for vitamin C testing of fruits and other coloured foods as it does not have these drawbacks and thus generally yield reproducible data.

The reported oxidising ability of vitamin C especially in alkaline medium requires for it to be stabilised during analysis for accuracy. Stabilizers are used as extractants and should be acidic with a high ionic strength to efficiently stabilise the vitamin while simultaneously prevent protein precipitation of the system. One such recommended solvent in vitamin C determinations is the oxalic acid (Okiei *et al*., 2009). Thus for a more efficient titrimetric analysis of vitamin C in fruits, the N-bromosuccinimide and oxalic acid are used as titrant and stabilizer respectively.

***Principle***

The procedure entails two redox reactions. In the first reaction, N-bromosuccinimide in the presence of potassium iodide and starch readily oxidises aqueous solutions of L-ascorbic acid to dehydro ascorbic acid while the NBS is irreversibly reduced to succinimide with the formation of hydrogen bromide. Completion of the first reaction, initiates the second where any small excess of added NBS, liberates iodine from the potassium iodide in the mixture to form a blue – black complex with starch (added as an indicator) indicating the end point of the titration.

### 2.3.3 Phytochemical composition

Plants constitute a myriad of chemicals which though non-nutritive, are known to be biologically active and have associated pharmacological/toxicological importance. Thus, these chemicals generally referred to as phytochemicals, are known to provide health benefits beyond basic nutrition when adequately included in diets (Liu, 2003). They may be defined as secondary metabolites Although many remain unidentified, some researchers group the known thousands under six main headings viz., alkaloids, terpenes, phenols, sterols,

carotenoids and organosulphurs based on differences in chemical structure and function (Chen and Blumberg, 2008) with others including fibre to make seven groupings (Erdman Jnr. *et al*., 2007). A number of qualitative protocols have been established for the general detection of the six groups of phytochemicals and these are universally acceptable for preliminary screening studies (Tiwari *et al*., 2011).

Generally, in assessing the health potential of fruits, the first four groups and on occasion, carotenoids, are tested (Wolfe *et al*., 2012; Etebu *et al*., 2012). Of these, phenolic compounds are postulated to be the most prominent in terms of their potential antioxidant activity and associated human health benefits in most species (Mariod *et al*., 2012) making them a necessary determinant when studying the AOA of plant foods. According to Erdman Jnr. *et al*. (2007) more than 8000 phenolics have been identified and they are generally subdivided into tannins, flavanoids, coumarins, stilbenes and phenolic acids.

***Test for total phenolics***

The colorimetric Folin-Ciocalteau procedure is usually employed in the general test for total phenolic compounds. This is so because it is fairly easy-to-use and has been time-tested to be efficient and reproducible (Yusoff and Iwansyah, 2011). The Folin-Ciocalteau reagent comprises two active compounds, phosphotungstic and phosphomolybdic acids. The protocol employs a reduction – oxidation mechanism in which the active compounds are reduced by the inherent phenolics in the test sample to blue-coloured tungsten and molybdenum respectively. The two compounds impart their blue colouration to the resultant mixture which has maximum ultraviolet-visible spectrum absorption at 750 nm. The obtained absorbance is considered proportional to the inherent total phenolic compounds in the test sample (Yusoff and Iwansyah, 2011).

### 2.3.4 Dietary fibre (df)

Dietary fibre is grouped into two fractions; soluble and insoluble with the total dietary fibre usually being employed for labelling purposes. There is established inverse relations between frequent consumption of fibre-rich foods and known chronic diseases including diverticulosis, appendicitis, haemorrhoids and some coronary heart diseases as well as other derived benefits such as prevention of constipation (Champ *et al*., 2003; Anderson *et al*., 2009; 2010; DeVries, 2013). The importance of dietary fibre to human health and nutrition has led to researchers advocating for the increased consumption of fruits with high dietary fibre contents for optimum health benefits (FAO/WHO, 2004). The definition for dietary fibre has evolved over the years with evolving scientific research methods, physiological and nutritional knowledge as well as influence from the interests of the food industry (Champ *et al*., 2003). Notwithstanding, there is ongoing scientific arguments as to the appropriate definition to adopt for the food industry (Anonymous, 2007).

Dietary fibre may now be defined as a complex mixture of organic constituents of foods especially those of plant origin that are measurable with a reasonably easy-to-follow protocol, non-digestible by enzymes in the human digestive tract but may be digested by microflora in the large intestine and which have one or more physiological effects associated with their intake (Champ *et al*., 2003; Anonymous, 2007).

In this context, dietary fibre includes celluloses, hemi-celluloses, gums, pectin, lignin, resistant dextrins, resistant starches and non-digestible oligosaccharides. To adequately quantify all these components with just one method has been proven impossible and thus a couple of standard procedures have been identified as adequate vis-a-vis the specific constituents targeted and/ or the type of food under study (Champ *et al*., 2003; Anonymous, 2007). A list of some methods and what they measure with respect to human nutrition is outlined in Table 2.1.

**Table 2.1: Major methods of dietary fibre analysis and what they measure in relation to human health and nutrition**

| NAME OF METHOD | NATURE OF PROTOCOL | OUTPUT |
|---|---|---|
| Total dietary fibre (or AOAC 985.29) | Enzymatic-gravimetric method | Soluble and insoluble polysaccharides and part of RS3 but not inulin/Polydextrose |
| Total dietary fibre (or AOAC 991.43) | Enzymatic-gravimetric method | Same as AOAC 985.29 but measures part of inulin unless corrected. Soluble and Insoluble dietary fibre can be quantified separately |
| AOAC 2000.11 | High Performance Liquid Chromatography (HPLC) | Polydextrose |
| Englyst method | Enzymatic – chemical or HPLC/GLC | Non-soluble polysaccharides |
| AOAC 995.16; AACC32-23 | Enzymatic | β – Glucans |
| Resistant starch | Enzymatic | Resistant starch |
| AOAC 997.08 | Enzymatic and Ion-exchange chromatography | Fructans |

Source: Champ *et al*. (2003)

Key: AOAC, Association of Official Analytical Chemists; AACC, Association of Cereal Chemists; GLC, gas liquid chromatography

The AOAC 991.43 as described by Lee *et al*. (1992) although it is quite an expensive procedure, has been suggested to be adequate for quantifying the various dietary fibre fractions in raw and processed fruits, vegetables and cereal grains (Anonymous, 2011).

***Principle underlining AOAC 991.43***

The protocol is applied to dried samples (duplicates which are defatted if fat content is greater than 10%) which are sequentially digested with the digestive enzymes of the gastrointestinal tract viz., α-amylase, amyloglucosidase and protease (Anonymous, 2011). The mixture is treated with ethanol to precipitate moderately soluble non-starch polysaccharides and the resultant insoluble fraction plus the ethanol-precipitated fraction is gravimetrically quantified. The protocol recovers lignin, celluloses, hemicelluloses, pectins, other non-starch polysaccharides and some resistant starch (specifically, some portions of

RS3) (Annonymous, 2007; Champ *et al*., 2003). Here, inulin is usually removed with inulinase to prevent it from being partially determined and the analysis corrected for ash and protein (Anonymous, 2011; Anonymous, 2007).

From this review, it can be inferred that inspite of the associated health benefits of plant bioactive compounds, there is no published data on the dietary fibre fractions (by approved methods for nutritional/ health claims) of the edible portions of *Annona muricata, Annona squamosa, Artocarpus altilis* and *Irvingia gabonensis.* There is also scant data on their total antioxidant activity and phytochemical constituents. This absence of extensive research (exacerbated by varietal differences associated with the species) makes the available information inadequate for generalising claims on their associated health potential. Thus, there is the need for further analysis especially from other indigenous sources to assess these health potential constituents of the afore-mentioned fruits.

# CHAPTER THREE

## 3.0 Materials and methods

### 3.1 Source of materials

Commercially mature fruits of *Irvingia gabonensis* and *Artocarpus altilis* were respectively obtained from trees at the Department of Horticulture and Queens Hall of the Kwame Nkrumah University of Science and Technology (KNUST) campus whereas that of *Annona muricata* and *Annona squamosa* were purchased from fruit stalls in Kumasi and Accra.

*Annona squamosa* (sweetsop)

*Artocarpus altilis* (breadfruit)

*Annona muricata* (soursop)

*Irvingia gabonensis* (African mango)

**Fig 3.1: Commercially matured fruits obtained from Kumasi and Accra**

### 3.2 Experimental procedure

The study was conducted in two phases.

**Phase 1**

***Sampling and preliminary sample preparation***

Fruits were obtained from the respective places (indicated above). Five healthy-looking breadfruits with average weight of 1266.84 g per fruit were selected and transported to the laboratory. They were immediately washed under running water and two unbruised fruits hand-picked for further preparation and use.

Sixty healthy-looking mature but unripe fruits of African mango were selected randomly for the study. These were stored in a paperboard for about 72 hours for them to ripen. The protocol was followed to avoid insect infestation during ripening on the tree. The ripened fruits were washed under running water in the laboratory and then sorted/graded by hand to select forty unbruised and infection-free ones (with average weight of 195.18 g per fruit) for further preparation and use.

Seven healthy-looking and unbruised soursop fruits (average weight of 414.75 g) were selected for the study. They were then washed under running water in the laboratory and four unbruised ones, hand-picked for further preparation and use.

Seventeen healthy-looking and unbruised mature but unripe sweetsop fruits were selected and kept for about 72 hours in ambient air to ripen. The ripened fruits were washed under clean running water in the laboratory and then sorted/graded by hand to select fourteen unbruised and ripe ones (with an average weight of 177 g per fruit) for further preparation and use.

The sampling for all the fruits was repeated – one for samples that were freeze-dried prior to use and the other for those used in the proximate determinations.

***Further sample preparation***

All washed samples were tissue-dried and weighed. They were then peeled to obtain the edible pulp. The pulp of African mango and breadfruit was sliced with a laboratory slicer to an approximate thickness of 0.2 mm. The seeds of *Irvingia sp* were obtained from the nut after removing the pulp and then its thickness reduced to about 0.2 mm with a stainless steel knife. The prepared samples were immediately bagged in pre-weighed zip-lock pouches and their weights (approximately 50 g) recorded.

Samples to be freeze-dried were frozen for 2 - 5 days at -20 $^{o}$C prior to freeze-drying and those for proximate determinations were immediately analysed**.**

**Freeze-drying**

The prepared samples were freeze-dried in a vacuum freeze-drier (model YK – 118, True Ten Industrial Co. Ltd, Taiwan) for 44 hours prior to the laboratory determinations. The freeze-dried samples were then milled (Thomas scientific mini-miller; Model 3383-L70), sieved with an impact lab test sieve of pore-size 400/425 microns (Model BS410 – 1:2000) and bagged in zip-lock pouches. About 50 g of the bagged samples were stored under refrigeration conditions (about 8 $^{o}$C) for the dietary fibre analysis and the rest stored in a commercial freezer (-20 $^{o}$C) for the other laboratory analysis.

**Phase 2**

The various determinations were carried out on the freeze-dried and stored samples from phase one.

**3.3 Determination of Antioxidant Activity (AOA) of Samples**

The analysis was done at the Chemical Laboratory of Noguchi Memorial Institute, Ghana. The method described by Ghasemi *et al*. (2009) was followed. About 5 mg of each sample

was weighed using a weighing balance (Mettler Toledo.XP 105 Delta Range) into eppendorf tubes. About 1 ml of methanol was then added to each sample, vortexed (Vortex V-1 Plus BM BIO) and then sonicated (Astrason $^{TM}$ Ultrasonic Cleaner Model) for complete dissolution of active compounds. The solution was decanted and the supernatant hereafter referred to as extract, used for the determinations.

About 100 µl of each extract was placed in a 96 well plate. Butylated hydroxytoluene (BHT) in concentrations of 0.625, 1.25, 2.5, 5, 10 and 20 mM was placed in another column as standard. About 100 µl of 0.5mM DPPH was pipetted into the well plate containing the extracts and different concentrations (aforementioned) of BHT. The well plate was covered with a foil and then gently shaken for about 30 seconds with the palm on a hard flat surface. The plate was incubated in the dark (placed in a clean empty drawer) for 20 minutes. Absorbance was read at 517 nm using a spectrophotometer (Infinite M200PRO) and the AOA calculated as a percentage of the reagent. The scavenging activity (AOA) was calculated as:

$$\frac{\text{Absorbance of blank} - \text{Absorbance of extract}}{\text{Absorbance of blank}} \times 100$$

**3.4 Determination of total phenolics**

The analysis was done at the Chemical Pathology Laboratory of Noguchi Memorial Institute, University of Ghana. The colorimetric Folin-Ciocalteu method described by Waterhouse (2002) was adopted to determine the total phenolic contents of each extract. Approximately 2 µl of each extract (from the determination of AOA) was aliquot into eppendorf tubes and diluted with 158 µl of milliQ water. Folin-Ciocalteau reagent (10 µl) was added to the solution and thoroughly mixed by vortexing. The solution was incubated at room temperature

for a maximum of eight minutes, 30 µl of $Na_2CO_3$ solution added and the mixture incubated at room temperature for two hours. The solution was aliquot into well plates and the absorbance read at 750 nm using an Infinite M200PRO TECAN spectrophotometre. The absorbance was compared to a standard curve of different gallic acid concentrations and the total phenolic content expressed as milligrams of gallic acid equivalent (GAE) per 100 g of edible portion of dried fruit.

### 3.5 Phytochemical screening

The phytochemical screening was done at the Phytochemistry Laboratory of the Department of Pharmacognosy, Kwame Nkrumah University of Science and Technology, Ghana. A number of standard preliminary testing protocols were followed.

#### 3.5.1 Detection of alkaloids

The method as proposed by Trease and Evans (2002) was adopted.

***Extraction of bioactive compounds***

Ammoniacal alcohol extracts of the various samples (about 0.5 g each) were evaporated to dryness and the residue extracted with dilute sulphuric acid and then filtered. The filtrate was made alkaline with dilute ammonia solution and the resultant solution again extracted with chloroform. The organic portion was evaporated off and the residue dissolved in dilute sulphuric acid.

***Mayer's test***

To about 2 ml of each extract, one drop of Mayer's reagent was added and the mixture observed for buff precipitate formation.

***Dragendorff's Test***

To about 2 ml of the various extracts, one drop of Dragendorff's reagent was added and the mixture observed for an orange-red precipitate.

### 3.5.2 Detection of glycosides

***General test for glycosides - Test for reducing sugars (Fehling's test)***

About 0.2 g of each sample was extracted with about 5ml of dilute HCL on a water bath for 2 minutes. The filtrates were made alkaline with 20% sodium hydroxide and then 1ml of Fehling's solution A and B added. The solution was heated on a water bath for 2 minutes and observed for colour of precipitate formed (Tiwari *et al*., 2011).

***Cyanogenic glycosides***

The method as proposed by Trease and Evans (2002) was followed. About 0.2 g of each sample was moistened (left no free liquid in the bottom of the flask) with few drops of distilled water. A strip of sodium picrate (2, 4, 6-trinitrophenate) paper was suspended in the neck of each flask and the flask warmed gently for about 2 minutes on a water bath. A change of colour of the sodium picrate paper to reddish-brown indicated the presence of cyanogenic glycosides.

***Cardiac glycosides***

The method as described by Onike (2010) was followed.

***Extract preparation***

Alcoholic extracts of 0.5 g of each sample was diluted with 10 ml of distilled water. To each of the diluted extract, 0.5 ml of strong lead acetate was added while shaking (for about 15 seconds) and then filtered with Whatman No. 1 filtre paper. About 10% of sulphuric acid was added drop-wise until no further precipitate formed. The solution was again filtered (with Whatman No. 1 filtre paper) and the filtrate, shaken (for about 10 seconds) with two successive 5 ml portions of chloroform. The two chloroformic extracts were combined and washed with 1ml of distilled water to remove lead unprecipitated ions. The chloroform was separated and filtered through a small plug of cotton wool. The extract was divided into two test portions, "**a**" and "**b"**.

***Kedde test***

Test portions **'a'** of each extract was evaporated to dryness, one drop of 90% alcohol and two drops of 2% 3, 5-dinitrobenzoic acid in 90% alcohol, added successively. The solution was made alkaline with 20% sodium hydroxide and observed for a purple colour formation which indicated the presence of α, β unsaturated – γ – lactone ring in the cardenolide.

***Keller – Kiliani test***

Test portions **'b'** of each extract was evaporated to dryness. To each residue, 0.4 ml glacial acetic acid containing traces of ferric chloride was added and the solution transferred into test tubes. About 0.5 ml concentrated sulphuric acid was carefully added down the side of each tube. The formation of a brown ring at the interface and a greenish colouration (with a taint of blue) in the upper acetic layer indicated the presence of 2-deoxy sugars.

### 3.5.3 Test for saponins

Using the froth's test, samples were extracted with distilled water in test tubes, filtred (with Whatman No. 1 filtre paper) and their filtrates vigorously shaken. The formation of a froth which did not readily break on standing for about 5 minutes indicated the presence of saponins (Trease and Evans, 2002).

### 3.5.4 Test for Tannins

The method as reviewed by Tiwari *et al*. (2011) was followed.

***Extract preparation***

About 25 ml of distilled water was added to 0.5 g of each sample and boiled for 5 minutes. The mixture was filtered using Whatman No. 1 filtre paper and the volume of the filtrate (extract) adjusted to 25 ml with distilled water.

***Lead acetate Test***

About 1 ml of each extract was diluted with 10 ml of distilled water and 2 to 10 drops of 1% lead acetate solution added. The solution was then observed for the formation of a precipitate which indicated the presence of tannins.

***Ferric chloride Test (True Tannins)***

About 1 ml of each extract was diluted with 10 ml of distilled water and 2 to 10 drops of 1% ferric chloride solution added. The formation of a blue-black precipitation indicated the presence of hydrolysable tannins whereas a brownish-green precipitation indicated the presence of condensed tannins.

### 3.5.5 Test for sterols

Chloroformic extracts of each sample were prepared. To about 5 ml of each extract in a test tube, 3 - 5 drops of acetic anhydride was first added and then about 2 ml of concentrated sulphuric acid added carefully down the side of the tube for the formation of a lower layer. The interface was observed for a bluish-green colour which indicated the presence of sterols (Trease and Evans, 2002).

### 3.5.6 Test for triterpenoids

Chloroformic extracts of each sample were prepared. To about 5 ml of each extract in a test tube, about 2 ml of concentrated sulphuric acid was carefully added down the side of the tube to form a lower layer. The formation of a reddish-brown/cherry red colour at the interface indicated the presence of triterpenoids (Trease and Evans, 2002).

### 3.5.7 Test for flavanoids

To about 5 ml of ethanolic extracts of each sample in test tubes, few drops of dilute ammonia was added gently by the sides. The mixture was then observed for yellow colouration which indicated the presence of flavanoids (Trease and Evans, 2002).

### 3.5.8 Test for coumarins

Chloroformic extracts of about 0.2 g of samples were evaporated to dryness and their respective residues dissolved in pre-heated distilled water and cooled. The cooled solution was divided into two test portions, A and B. To the test portion A of each extract, about 0.5 ml of 10% ammonia solution was added. Test portions A and B for each extract were observed under UV light for the occurrence of an intense bluish green fluorescence which indicated the presence of coumarins (Trease and Evans, 2002).

## 3.6 Proximate Determinations

The proximate analysis was done on the fresh fruit samples. The analysis was done at the Biochemistry Laboratory of Crop Research Institute, Centre for Scientific and Industrial Research, Ghana.

### 3.6.1 Determination of moisture content

Approximately 2.0 g of each fresh sample was weighed into a previously dried and weighed petri-dish. The petri dishes with samples were then dried in a thermostatically controlled oven (OSK electric oven) at 105°C overnight to a constant weight. The petri-dishes were removed and transferred into a desiccator for cooling after which they were weighed. Moisture content was determined by difference and expressed as a percentage (AOAC, 1997).

### 3.6.2 Ash determination

About 2.0 g of dried sample (from moisture determination) was weighed into a pre-ignited and previously weighed porcelain crucible. To ash, the crucible with sample was placed in a muffle furnace and ignited for 2 hours at 600 °C. They were then cooled to about 105 °C in a forced convection oven and further cooled to room temperature in a desiccator. The crucibles

and their contents were weighed. The ash content was determined by difference and expressed as a percentage (AOAC, 1997).

### 3.7.3 Determination of crude fat

About 2.0 g of the dried sample from the moisture determination was ground and transferred into a filter paper bag and placed into a thimble holder. 200 ml of hexane was measured into a previously dried and weighed round-bottom flask and this was assembled together with the thimble holder and its contents. The Quickfit condenser was connected to the Soxhlet extractor and refluxed for 16 hours on low heat on a heating mantle. The flask was then removed and the solvent evaporated in a steam bath. The flask containing the fat was heated at 105°C in an oven for 30 minutes, cooled in a desiccator and the weight of the collected fat determined and expressed as percentage crude fat (AOAC, 1997).

### 3.7.4 Determination of crude fibre

Approximately 2.0 g of defatted samples were transferred into a 250 ml Erlenmeyer flask and 0.5 g of asbestos added. 200 ml of boiling 1.25% sulphuric acid ($H_2SO_4$) was added to the flask and immediately transferred onto a hot plate. A cold finger condenser was attached to it and the sample (entirely made wet with solvent) boiled for 30 minutes. After 30 minutes, the flask was removed and its contents filtered through linen cloth placed in a funnel. The residue was washed with boiling water until the washings were no longer acidic (by testing with blue litmus paper). The sample with asbestos was then washed back into the flask with 200 ml of boiling 1.25% sodium hydroxide (NaOH) solution.

The flask was reconnected to the condenser and boiled for 30 minutes. The contents were filtered through a linen cloth in a funnel, thoroughly washed with about 300 ml of boiling water and then with 15 ml of alcohol. The residue was transferred into a previously dried and weighed porcelain crucible. Drying of crucible was done in an oven at 100 °C for 1 hour and

then cooled in a dessicator. The crucible and its contents were ignited in an electric furnace at 600 °C for 30 minutes, cooled and reweighed. The loss in weight was reported as percentage crude fibre (Kirk and Sawyer, 1991).

### 3.7.5 Determination of crude protein

The micro- Kjedahl method as described by Kirk and Sawyer (1991) was adopted.

***Digestion***

To about 35 mg of ground sample in a digestion tube, 1 g catalyst mixture, 2 ml of 30% hydrogen peroxide and 2 ml concentrated sulphuric acid were sequentially added. The sequence was followed to avoid spattering. The mixture was allowed to digest for 30 minutes and then cooled. About 200 ml of deionised water was added to dissolve the digest residue and the resultant solution allowed to cool at room temperature.

***Distillation***

The digest solution was then transferred into a cleaned Kjedahl distillation apparatus. It was ensured that no residue was left in the digestion flask by rinsing it twice with about 2 ml of deionised water. 6 ml of boric acid solution plus three drops of indicator solution were put in a 250 ml Erlenmeyer flask and then placed beneath a condenser. The condenser tip was adjusted to extend well below the surface of the solution. To distil the ammonia, 8 ml of sodium hydroxide-sodium thiosulphate solution was then added to the digest in the distillation apparatus and distillation allowed to continue until about 50 ml of distillate was collected.

***Titration***

The distillate was titrated against 0.02 N hydrochloric acid (HCl) solution until first appearance of violet.

For blank, same quantity of reagents and followed procedures for digestion, distillation and titration as afore elaborated for samples, was used.

***Calculation***

Percent nitrogen and protein of each sample was calculated as per the formulae below;

$$\% \text{ Crude nitrogen} = \frac{(\text{ml HCl in sample} - \text{ml blank}) \times \text{normality HCl} \times 14.007 \times 100}{\text{mg of sample}}$$

% Crude protein = % N × 6.25 (conversion factor)

### 3.7.6 Carbohydrate content

The percent carbohydrate of each sample was determined by difference from the sum of the other five determinations (moisture, crude fibre, crude protein, ash, crude fat) and 100%.

### 3.8 Vitamin C determination

The vitamin C analysis was carried out at the Nutrition Laboratory of the Department of Nutrition and Food Science, University of Ghana. The method as described by Okiei *et al*. (2009) was followed.

***Extraction of ascorbic acid in samples***

About 5 g of each sample was blended with 100 ml of 20% trichloroacetic acid (TCA) diluted twofold with 0.5% oxalic acid solution (33.3 ml TCA + 66.6 ml oxalic acid). The mixture was transferred into a volumetric flask and topped up with the oxalic acid solution to 250 ml. The contents were well mixed, allowed to settle and then filtred using Whatman's No. 1 filtre paper.

***Preparation of blank***

To 17 ml of 0.5% oxalic acid in a volumetric flask, 3 ml of 20% TCA was added and the resulting solution used as blank.

***Ascorbic acid content***

About 5 ml of each extract and blank was pipetted into labelled 100 ml beakers. About 5 ml of 4% potassium iodide, 2 ml of 3% acetic acid and 3 drops of 1% starch indicator were sequentially added and mixed. The resultant solutions were individually titrated against 0.01% N-bromosuccinamide solution with continuous shaking until a permanent bluish-purple colour was observed and the end point of titration noted.

The vitamin C content (mg percent) of each extract was calculated as,

$$(\text{Test Titre} - \text{Blank titre}) \times 0.1 \times 0.99 \times \frac{100}{\text{W}} \times \frac{\text{Total volume of extract}}{\text{Total volume of extract used}}$$

Where, W, is the weight of each sample used.

**3.9 Determination of dietary fibre fractions**

The analysis was done by the Nutritional Chemistry and Food Safety Laboratory of the Covance Laboratories, USA.

The soluble and insoluble dietary fibre fractions were determined by the enzymatic-gravimetric method officially known as AOAC method 991.43 (AOAC, 1995) and all samples used were freeze-dried and refrigerated (approximately 8 $^{o}$C) prior to the analysis. With each assay, two blanks were run along with samples. Approximately 1g of each sample (duplicate) was weighed into 400 ml tall-form beakers and 40 ml MES-TRIS buffer solution plus magnetic stirring bar, added. The solution was stirred until samples were completely dispersed in solution. About 50 µL heat-stable α-amylase solution was then added while stirring at low speed.

The beakers were covered with aluminium foil squares and placed in shaking water bath at 95-100 °C and then incubated for 35 minutes with continuous agitation after which they were removed from the water bath and cooled to 60 °C. The foil covers were then removed and

any rings around beaker, scrapped into bottom of beaker with spatula. The side wall of beaker and spatula were then rinsed with 10 ml distilled water using a pipette. About 100 µL protease solution was added to each of the sample, covered with Aluminium foil, and incubated in a water bath at 60±1 °C for 30 minutes with continuous agitation. Samples were removed, 5 ml of 0.561 N HCL solution dispensed into sample while stirring, and the pH adjusted 4.1 – 4.8 with either 5% NaOH or 5% HCl solution. About 200 µl amyloglucosidase solution was then added while stirring on magnetic stirrer and the aluminium cover replaced. The resultant solution (enzyme mixtures), were then incubated in shaking water bath at 60 °C for 30 minutes, with constant agitation.

### 3.9.1 Insoluble dietary fibre fraction (IDF)

*Preparation of Crucible*

Celite was placed in crucibles and the crucible containing celite, tarred to the nearest 0.1 mg. The bed of celite was redistributed in crucible using about 3 ml of distilled water. A suction pump was then applied to the crucible to draw celite onto the fritted glass as even mat.

Enzyme mixture (afore described) was filtered through already prepared crucibles into a filtration flask. The residue was washed twice with 10 ml distilled water preheated to 70°C. The filtrate and water washings were both saved for later use. The solution was transferred to a pre-tarred 600 ml tall-form beaker. The residue was again sequentially washed twice with 10 ml of 95 % ethanol and acetone. The crucibles containing residue, were dried overnight in 103°C oven after which they were cooled in a desiccator for about 1 hour. Crucibles containing dietary fibre residue and celite were weighed to nearest 0.1 mg and the actual residue weight determined.

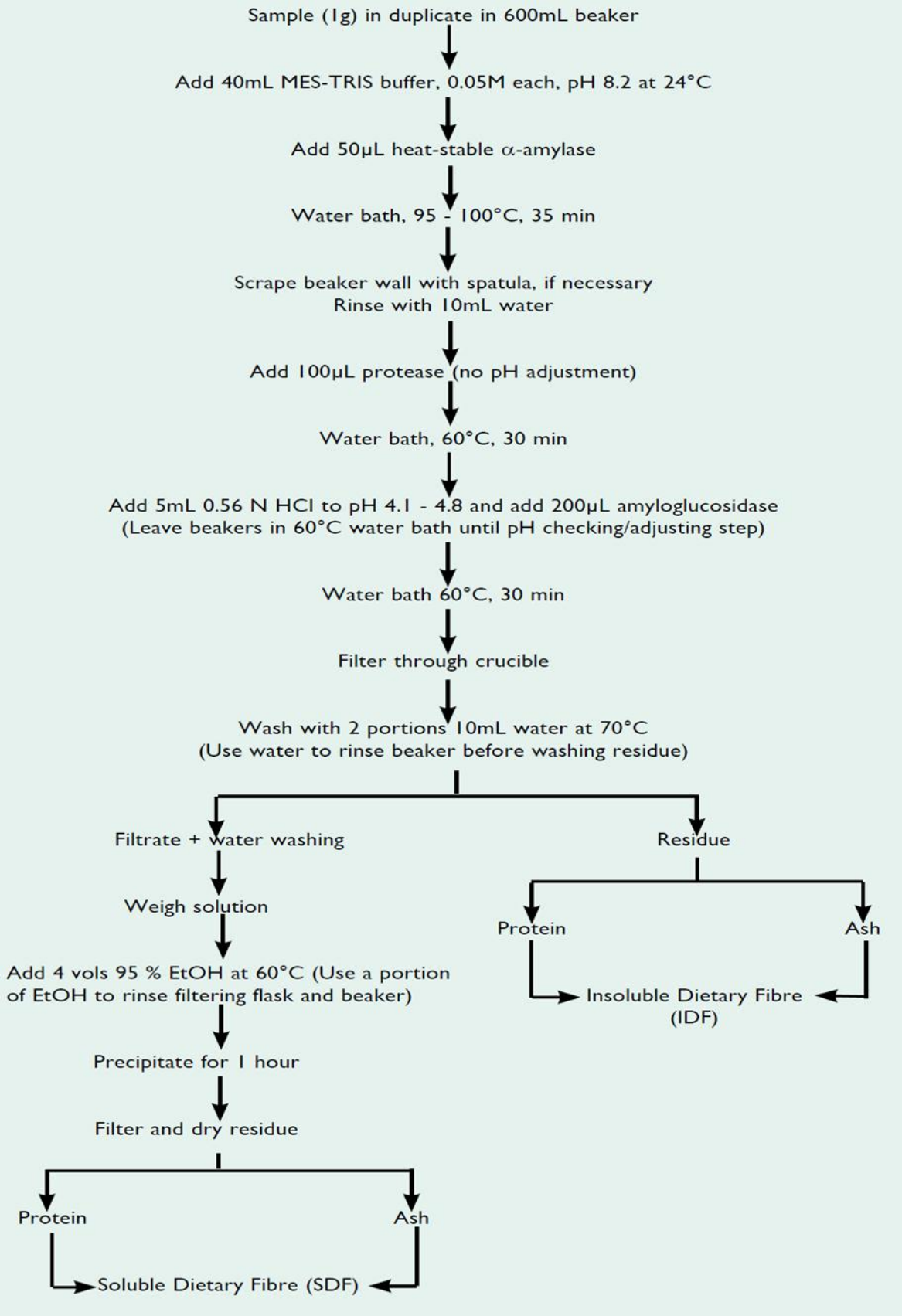
One residue was then analysed for protein, and the second residue of the duplicate, analysed for ash.

### 3.9.2 Soluble dietary fibre (SDF) fraction

*Preparation of crucible*

Crucibles containing celite were tarred to nearest 0.1 mg, wetted and redistributed using 15 ml of 78% ethanol from wash bottle. Suction was then applied to the crucible to draw celite onto fritted glass as an even mat.

The stored combined solution of filtrate and water washings from the IDF determinations, were weighed in pre-tarred beakers, four volumes of 95% ethanol added and then preheated to 60°C. A portion of ethanol was used to rinse filtering flask used in IDF analysis. Precipitate was allowed to form at room temperature for 60 minutes. The precipitated enzyme digest was filtered through already prepared crucibles. Using a wash bottle with 78 % ethanol and a rubber spatula, remaining particles were quantitatively transferred into the crucible and the residue successively washed using a vacuum with two 15 ml portions of 78 % ethanol, 95 % ethanol and acetone. Crucibles containing residue were dried overnight in 103°C oven after which they were cooled in desiccator for approximately 1 hour. They were then weighed to nearest 0.1 mg and the actual residue weight determined. One residue was then analysed for protein, and the second residue of the duplicate, analysed for ash.




Source: Anonymous (2011).

**Fig 3.2: Analytical scheme for determination of dietary fibre fractions of fruit samples**

### 3.10 Statistical Analysis

Data obtained were analysed using the one-way Analysis of Variance (ANOVA) and Pearson's correlation coefficient by employing statgraphics and graphpad softwares**.**

# CHAPTER FOUR

## 4.0 Results and Discussion

### 4.1 Proximate composition (% mean) of underutilised fruits

The proximate composition of the samples on dry weight basis is outlined in Table 4.1. The fruits generally had high moisture contents which ranged from 40.20% for African mango seeds (hull included) to 84.07% for African mango pulp. They also had considerable protein content with values from 6.71% for Africa mango seeds to 2.63% for its pulp. Fat and fibre contents ranged from 2.60% (breadfruit) to 13.90% (African mango seeds) and 2.09% (breadfruit) to 13.51% (African mango seeds) respectively.

**Table 4.1: Proximate composition (% mean) of edible portions of four underutilised fruits**

| **Proximate** | **Samples** | | | | |
|---|---|---|---|---|---|
| | African mango pulp | African mango seeds | Breadfruit pulp | Soursop pulp | Sweetsop pulp |
| Moisture | $84.07\pm0.51^{d}$ | $40.20\pm3.69^{a}$ | $67.89\pm0.51^{b}$ | $82.37\pm0.26^{d}$ | $73.19\pm0.81^{c}$ |
| Ash | $2.97\pm0.47^{b}$ | $2.44\pm0.07^{a}$ | $2.64\pm0.02^{ab}$ | $4.00\pm0.03^{c}$ | $3.07\pm0.25^{b}$ |
| Protein | $2.63\pm0.11^{a}$ | $6.71\pm0.07^{d}$ | $2.87\pm0.14^{a}$ | $4.61\pm0.36^{c}$ | $3.26\pm0.18^{b}$ |
| Fat | $2.84\pm0.17^{ab}$ | $13.90\pm0.58^{d}$ | $2.60\pm0.41^{b}$ | $3.55\pm0.09^{c}$ | $3.30\pm0.17^{ac}$ |
| Crude fibre | $4.15\pm0.34^{a}$ | $13.51\pm2.15^{b}$ | $2.09\pm0.29^{c}$ | $4.81\pm0.33^{a}$ | $4.35\pm0.12^{a}$ |
| Carbohydrates | $3.33\pm0.91^{a}$ | $23.24\pm5.20^{c}$ | $21.92\pm0.78^{c}$ | $0.65\pm0.29^{a}$ | $12.83\pm0.83^{b}$ |

Means in the same row with different superscript letters are significantly different ($P < 0.05$)

Moisture content of foods gives an indication of the available dry matter as well as plays a major role in determining the propensity of the food to spoil (Appiah *et al*., 2011). The high moisture content as observed in the *Annona species* and African mango pulp reflects the limited shelf-life of these climacteric fruits. When harvested at physiological maturity and stored at room temperature, sweetsop with an obtained moisture content of 73.19% can last for 2 to 2.5 days, soursop (82.37%) for five days (Lima and Alves, 2011) and African mango pulp (84.07%) for about 5 days (Etebu, 2012).

Analogous moisture values have been obtained in other studies for all the samples except for African mango seeds viz., 80% for African mango pulp (Onimawo *et al*., 2003), 67.86% for sweetsop (Folorunso and Modupe, 2007), 81.2% - 91.76% for soursop (Lim, 2012; Folorunso and Modupe, 2007) and 60.5% for breadfruit (Asibey-Berko and Tayie, 1999). Relatively low but varying moisture values including 4.0% (Oboh and Ekperigin, 2004) and 15.21% (Ekpo *et al*., 2007) for African mango seeds (without hull) as compared to the 40.20% obtained from this study have been reported. For this study, the analysis was done on unfermented seeds with hull whereas most of the cited research works were done on fermented seeds and often without the hull - a probable cause for the observed difference in values. Mooreover, Ladipo *et al*. (1998) suggested varietal differences in the African mango to be a major factor in varying fruiting phenology (July-August; August-September and February) – a phenomenon that has been suggested by Ekpo *et al*. (2007) to be a probable cause for significant differences in seed moisture content.

The obtained results agree with the reports by Ainge and Brown (2001) and Lima and Alves (2011) that suggest the potential use of soursop, sweetsop and African mango pulp in the commercial production of juices, jams and jellies. Considering the hot climate of Africa, the intake of these fruits just like oranges with moisture of 85% (Nair, 2011), will satiate many in the hot afternoons.

There were significant variations at $P < 0.05$ in ash contents for soursop and African mango (pulp and seeds) while breadfruit, African mango pulp and sweets had similar values. Of the fruits studied, soursop had the highest ash content with 4.00% while African mango seed had the least content with 2.44%. The ash content obtained for soursop was comparatively higher than the 0.89 - 0.90% reported by Amusa *et al*. (2003). This variation could be attributed to varietal differences and impact of type and composition of soil of fruit origin (Marinov *et al*., 2005). However, values obtained for African mango seeds (2.44%), breadfruit (2.64%) and sweetsop (3.07%) were comparable to that reported in other studies; 2.06% – 3.8% (Ekpo *et al*., 2007; Matos *et al*., 2009), 2.37% (Appiah *et al*., 2011) and 2.8% (Lim, 2012) respectively. Ash content for pulp and seeds of the African mango is also in harmony with studies by Ayivor *et al*. (2011) which showed significant composition of an array of minerals viz., aluminium, zinc, copper, cobalt, magnesium, manganese, chlorine, iron and iodine in both the mesocarp and endocarp of the African mango fruit in Ghana.

Crude ash gives an approximate measure of the total mineral composition of foods notwithstanding contaminations which may lead to higher than factual values. The study shows the studied fruits had higher ash composition than some common fruits including, 0.95% for avocado (USDA, 2009) and papaya with 1.32% (Mandle *et al*., 2012). In addition, sweetsop and soursop had higher ash contents than the (2.70 – 2.81) % for banana reported by Daramola *et al*. (2000).

Protein values varied considerably ($P < 0.05$) for the samples except for breadfruit and African mango pulp which statistically had similar protein contents. The highest protein content was observed in African mango seed with 6.71% while its pulp had the least protein content with 2.63%. Reported protein content of sweetsop (1.2 – 2.4) % by Pinto *et al*. (2005) and soursop 1.21% by Amusa *et al*. (2003) are at variance with values obtained in this research - 3.26% and 4.61% respectively - showing higher protein contents of the studied

varieties. Protein obtained for breadfruit (2.87%) corresponds to the reported 3.8% by Appiah *et al*. (2011) for some Ghanaian varieties. African mango seeds with a protein content of 6.71%, also falls within the range, 5.08% - 8.71%, reported by Ekpo *et al*. (2007), Onimawo *et al*. (2003) and Matos *et al*. (2009). Generally, the samples had protein contents comparatively higher than literature values for some common fruits. Significant among them as reported by Lozano (2006) include, 1.3% for banana, 0.9% for oranges and 0.3% for apples.

African mango seed with a fat composition of 13.51% had the highest fat composition out of the four fruits studied (with the least being observed in breadfruit) but the obtained value was comparatively lower than the 66.6%, 74.13% and 58.4% reported by Ekpe *et al*. (2007), Ekpo *et al*. (2007) and Oboh and Ekperigen *et al*. (2004), respectively. This shows the variety studied may not be viable for commercial production of oil as some others in literature but its other nutritional components afore-mentioned as well as its "drawability" can be tapped for industrial and pharmaceutical purposes (Ainge and Brown, 2001). It is noteworthy that the African mango seed fat constitutes myristic and lauric fatty acids as well as linolenic (omega 3) and linoleic (Omega 6) acids - fatty acids that are essential for human health (Leakay *et al*., 2005; Ainge and Brown, 2001).

There is varying data for the fat composition of soursop in literature with values ranging from zero (Not Detected) on fresh weights (Folorunso and Modupe, 2007); 0.87% on dry weight (Abbo *et al*., 2006) to 3.25% for its juice (Enweani *et al*., 2004). The value obtained for this study was 3.55% on dry weight showing a considerable fat content in the studied variety and supports studies whereby essential oils have been extracted from the fresh fruits of soursop with potential for human health (Dembitsky *et al*., 2011). The value obtained for breadfruit, 2.6%, is comparable to the 2.36% reported by Appiah *et al*. (2011) for some Ghanaian varieties. However, reiterating reports by Leakay *et al*. (2005) and Ainge and Brown (2001),

fruits predominantly constitute unsaturated fatty acids and generally lack the much dreaded trans-fats thus, the considerable fat contents obtained in the studied fruits may not pose any health threats to consumers.

The carbohydrate content of the samples varied significantly at $P < 0.05$ for soursop with the least value of 0.65%, African mango pulp (3.33%), and sweetsop (12.83%) while similar ($P < 0.05$) contents were obtained for African mango seeds with the highest value of 23.24% and breadfruit with 21.92% of carbohydrate composition. Reported carbohydrate content of soursop, 14.98%, by Amusa *et al*. (2003), is comparatively higher than the obtained value. The relatively high value obtained for sweetsop on the other hand, is in harmony with a study by Nwokocha and Williams (2009) suggesting the potential use of sweetsop flour as an industrial thickener.

African mango seed flour is widely used in most indigenous localities of the tree as food thickeners and also to impart viscosity in soups and sauces (Ekpo *et al*., 2007). Matos *et al*. (2009) attributed this thickening property largely to its carbohydrate content - an attribute that was evident in the studied variety with obtained carbohydrate content of 23.24%. The obtained carbohydrate value of 21.92% for breadfruit supports a suggestion by Ragone (2011) of its potential as a staple food in place of the imported carbohydrate rich staples such as rice and potatoes as well as the possibility of using it to partially replace wheat flour for most baked products in their indigenous areas. In addition, the high carbohydrate content of sweetsop, breadfruit and African mango seed, gives the underutilised fruits an edge over some common fruits such as mango with carbohydrate content ranging from 14.1% – 15.4% (Abdualrahman, 2013).

The fibre content of African mango seeds (13.51%), breadfruit (2.09%) and African mango pulp (4.15%) varied significantly ($P < 0.05$) from each other and from soursop (4.81%) and

sweetsop (4.35%) which were similar ($P < 0.05$). The obtained fibre for African mango seeds is at variance with the 1.9% reported by Ekpe *et al*. (2007) and 1.04% by Ekpo *et al*. (2007) but comparable to the 10.23% reported by Onimawo *et al*. (2003). The variation may be due to the differences in variety as well as the processing protocol (drying and size reduction methods), employed prior to the analysis and as is the case for this analysis, the inclusion of the seed hull in the determination (Anderson *et al*., 2010). Although there is scant literature on the African mango pulp, the obtained value, 4.2%, was comparatively higher than the 0.4% reported by Onimawo *et al*. (2003). Pinto *et al*. (2005) reported of comparatively lower values of crude fibre for sweetsop and soursop with values of up to 2.5% for sweetsop and 0.95% for soursop. Breadfruit with an obtained fibre content of 2.1% was lower than the reported value of 3.12% by Appiah *et al*. (2011) for some Ghanaian varieties. This further corroborates the report by Ragone (2011) of the varietal differences of the species even within a given locality thereby indicating unique potentials of each variety based on its composition.

According to Anderson *et al*. (2010), crude fibre content of foods gives an indication of its dietary fibre as it measures one-half to one-seventh of the total dietary fibre component of the food. Considering the health benefits of dietary fibre in diets as elaborated by Champ *et al*. (2003), the relatively high crude fibre content observed in the studied fruits is much desired. Compared with the fibre content of some common fruits viz., mango with values ranging from 1.6% – 4.5% and apple with 0.86 – 1.81% (Lim and Rabeta, 2013), the superiority of the studied fruits to some common ones is further brought to bear.

On the whole, the studied fruits were better than some common fruits in terms of their protein, ash, fat and carbohydrate contents further signifying their potential in contributing to the nutrient needs of malnourished communities.

### 4.2 Dietary Fibre Composition of studied fruits

Fig 4.1 shows the dietary fibre fractions obtained for the studied samples. The various fractions ranged from 8.01 g/100g (for breadfruit) - 18.00 g/100g (for African mango pulp), 2.28 g/100g (soursop) – 7.35 g/100g (African mango seeds) and 11.50 g/100g (soursop) - 22.70 g/100g (African mango pulp) for the insoluble, soluble and total dietary fibre contents respectively. The samples had high insoluble dietary fibre fractions in accordance with reports by Devries (n.d) and Wong and Jenkins (2007) that the insoluble dietary fibre forms about two-thirds of the total fibre of most foods. This observation is however contrary to the assertion by Anderson *et al*. (2010) that fruits are mainly soluble dietary fibre sources.

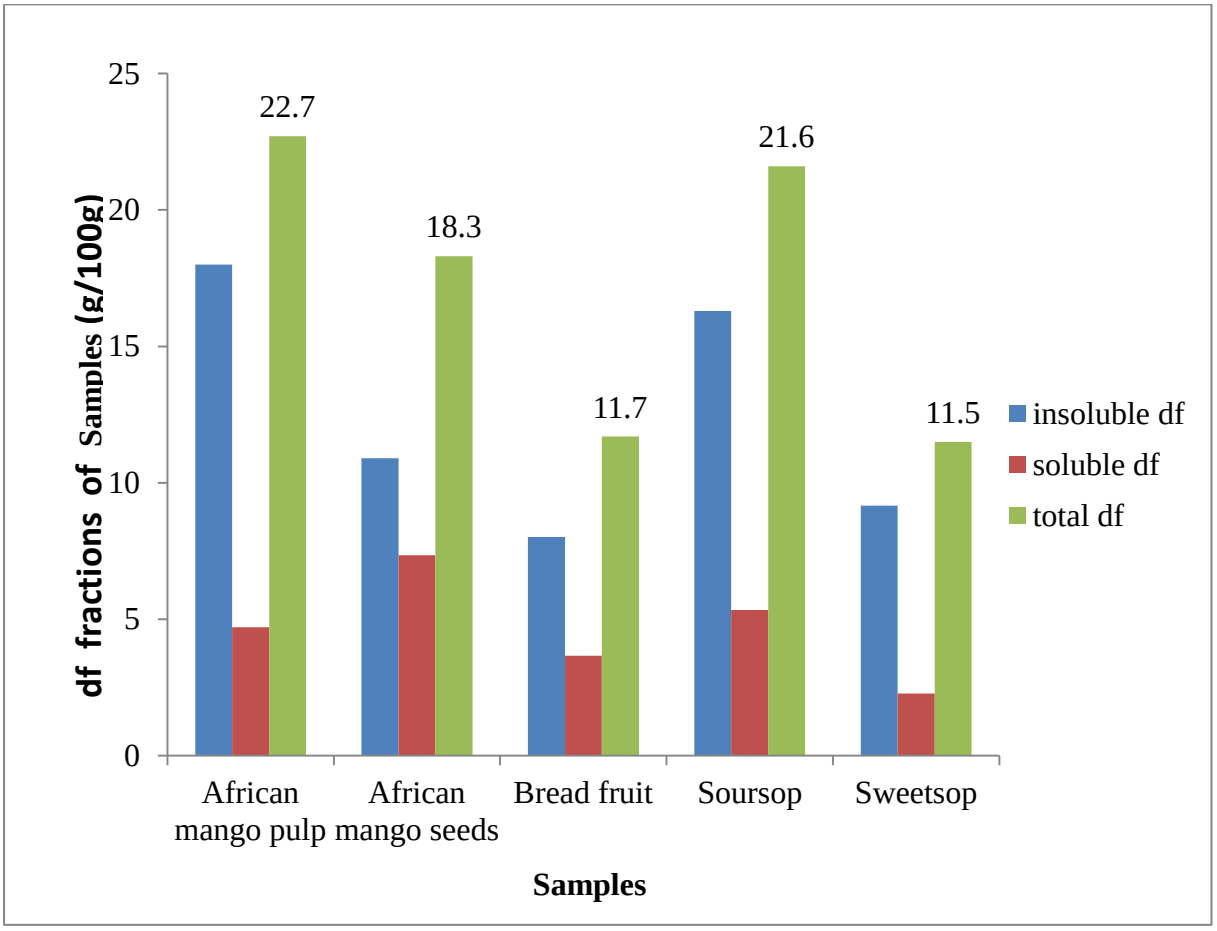



Fig 4.1: The dietary fibre fractions (g/100g) of four indigenous underutilised fruits

Associated health benefits of insoluble dietary fibre are many and prominent among them include the inverse relations between its intake and risk of diverticular disease, constipation,

haemorrhoids and appendicitis (Champ *et al*., 2003; DeVries, n.d). Foods noted as good insoluble dietary fibre sources as reported by Li *et al*. (2002) include whole grains such as yellow corn meal (with insoluble fraction of 3.32 g/100g), ready-to-eat corn tortilla beans (with 4.39 g/100g insoluble fibre), firm whole-wheat bread (5.21 g/100g) and some vegetables such as raw broccoli (with 3.06 g/100g insoluble fibre fraction). Comparing these values to that obtained for the studied fruits (least being 8.01 g/ 100g), suggest the potential of these underutilised fruits to contribute significantly to the insoluble fibre needs of consumers when incorporated into diets.

The underutilised fruits also had higher soluble dietary fibre fractions (2.28 to 7.35 g/ 100g) than that of some traditional commercial fruits viz., 0.58 g/ 100g for banana, 1.37 g/ 100g for oranges and 0.04 g/ 100g for pineapple (smooth cayenne) (Li *et al*., 2002). Anderson *et al*. (2009; 2010) recommends for a minimum intake of 6 g/day of soluble dietary fibre to achieve metabolic effects such as glycaemic control, lowering of 5.4% of LDL blood cholesterol and reduction in the risk of 9% of coronary heart diseases. In line with this report, the findings of this study indicate the potential of these fruits to significantly contribute to consumers meeting their soluble fibre needs. It also suggests the potential of these fruits to contribute to health when duly exploited. A point in case is the report by Ngondi *et al*. (2009). The researchers established the efficacy of the African mango seeds in combating obesity and maintaining blood glucose levels especially in diabetics. The same may be true for the other samples as they also had considerable soluble fibre fractions.

On the whole, the obtained total dietary fibre contents of the studied fruits (least being 11.50 g/ 100 g) were higher than what has been reported by Li *et al*. (2002) for the common ones on the market viz., banana with 1.79 g/100 g, pineapple with 1.46 g/100 g, apple (red delicious-skin included) with 2.21 g/ 100 g and pear with 3.16 g/ 100 g. The obtained total dietary

fibre contents of the samples also compared favourably to the recommended daily allowance (RDA) of dietary fibre for optimum health (Table 4.2).

In cognisance of the recommendation by the European Union (2006) suggesting foods labelled as "high in fibre" to contain at least 6 g/ 100g of total fibre, the studied underutilised fruits can be considered as high dietary fibre sources and further exploited for their associated benefits.

**Table 4.2: Recommended minimum intake for dietary fibre (g/day)**

| AGE/PHYSIOLOGICAL STATE | GENDER | |
|---|---|---|
| | MALE | FEMALE |
| 0 – 12 months | NOT DETERMINED | NOT DETERMINED |
| 1 – 3 years | 19 | 19 |
| 4 – 8 years | 25 | 25 |
| 9 – 13 years | 31 | 26 |
| 14 – 18 years | 38 | 26 |
| 19 – 50 years | 38 | 25 |
| 51 and older | 30 | 21 |
| Pregnant women | - | 28 |
| Lactating women | - | 29 |

Source: Anderson *et al*. (2010)

### 4.3 Phytochemical constituents of studied fruits

The detected phytochemicals of the studied samples are shown in Table 4.3. Tannins and glycosides were detected in all the samples while saponins were detected in all except sweetsop. Triterpenoids were also detected in all the samples except in soursop. Sterols and flavanoids were detected in three out of the five samples whereas coumarins were present in only breadfruit and soursop. Alkaloids were not detected in any of the samples.

**Table 4.3: Phytochemical constituents of the edible portions of four underutilised fruits**

| Phytochemicals | Fruits | | | | |
|---|---|---|---|---|---|
| | ***AMP*** | ***AMS*** | ***Breadfruit*** | ***Soursop*** | ***Sweetsop*** |
| **Tannins** | + | + | + | + | + |
| **Saponins** | + | + | + | + | - |
| **Sterols** | + | + | + | - | - |
| **Alkaloids** | - | - | - | - | - |
| **Glycosides** | + | + | + | + | + |
| - Cyanogenic | - | - | - | - | - |
| - Cardiac | - | + | - | + | + |
| **Flavanoids** | + | + | - | + | - |
| **Coumarins** | - | - | + | + | - |
| **Triterpenoids** | + | + | + | - | + |

(+) implies presence; (-) implies absence

The detection of tannins in the samples is in accordance with reports by Etebu (2012), Onyechi *et al*. (2012) and Adekunle and Oyerinde (2004). This gives an indication of the potential health benefits of the fruits as tannins are acclaimed for their free-radical scavenging activity and their antimicrobial, antiviral and anti-inflammatory properties (Akiyama *et al*., 2001). However, according to Etebu (2012) the astringent and bitter taste imparted by tannins to plant-based foods, generally makes them undesirable when present in very high quantities in ready-to-eat foods such as fruits. The reports, afore-mentioned, show the tannin content of these fruits when ripe is just adequate.

Saponins were detected in African mango (seeds and pulp), breadfruit and soursop in harmony with reports by Wolfe *et al*. (2010), Onyechi *et al*. (2012) and Etebu (2012) respectively. Saponins are common in most plants and have been postulated to have a wide range of biological activity including antioxidant, anticarcinogenic as well as having immunostimulant properties thereby exhibiting the potential to cure a number of diseases (Trease and Evans, 2002). It is noteworthy that although Francis *et al*. (2002) reported adverse effects of some types of saponins on fishes (and other aquatic lives), the researchers

also indicated the associated health beneficial properties (to humans) of those found in edible fruits thereby eroding any cause for alarm in the consumption of these fruits.

All the samples showed negative inferences to the test for alkaloids. Alkaloids have not been detected in sweetsop (EFSA, 2009) and breadfruit (Ragone, 2011) but have been detected in African mango seeds and pulp (Etebu, 2012) as well as in the mesocarp of soursop (EFSA, 2009). However, Etebu (2012) showed a decline in alkaloid content of African mango mesocarp with increasing storage duration suggesting the need for the analysis to be done on fresh samples for an adequate assessment.

Glycosides were detected in all the studied samples confirming similar reports by Wolfe *et al*. (2010), Onyechi *et al*. (2012) and Etebu (2012). Their detection indicates the existence of compounds with a pharmacologically active genin and at least one reducing sugar. Specific tests for cyanogenic glycosides had negative results for all the samples. It has however been reported to be present in minute amounts in sweetsop (Coronel, 1986). The absence of cyanogenic glycosides in the fruits is desired because of the toxicity associated with the intake of this compound although Onike (2010) suggested relaxant and calming effects by the chemical on the heart and muscles when it is consumed in small doses.

As reported in other studies (Dembitsky *et al*., 2011; Wolfe *et al*., 2010; EFSA, 2009), African mango seeds, soursop and sweetsop tested positively to cardiac glycosides. Usunomena (2012) reported the importance of cardiac glycosides in treating congestive heart failure thus, its presence in these fruits is much desired. Moreover, the findings support a review by Dembitsky *et al*. (2011) in which the researchers suggested the potential of soursop

and sweetsop in the treatment of cardiac-related ailments (as used in folkloric medicine) by analysing their cardiac glycoside composition.

Flavanoids were detected in African mango (pulp and seeds) and soursop. The finding corroborates earlier detections for some Nigerian varieties by Wolfe *et al*. (2010) for African mango seeds, Etebu (2012) for African mango pulp and Onyechi *et al*. (2012) for soursop. Flavanoids are acclaimed for their antioxidant and antimicrobial activity (Dembitsky *et al*., 2011) and Etebu (2012) suggests their significant contribution to the folkloric medicinal use of fruits. The absence of flavanoids in breadfruit and sweetsop is in agreement with reports of non-detection by Ragone (2011) for some breadfruit varieties in the Pacific Islands and EFSA (2009) for sweetsop from the Philippines.

A positive inference for coumarins was obtained for soursop and breadfruit. Adewole and Ojewole (2008) reported the presence of coumarins in soursop but there is dearth of data on its presence in breadfruit. Coumarins were not detected in sweetsop and African mango (both pulp and seeds) in the study. Coumarins are not usually assessed in the phytochemical analysis of most fruits (including those studied) (EFSA, 2009; Etebu, 2012; Wolfe *et al*., 2010) resulting in limited data on them. According to Trease and Evans (2002) however, coumarins are anti-stress compounds that are postulated to have antiviral (including anti-HIV) activity and also employed as oral-anticoagulants – an essential agent in the treatment of arterial thrombosis. Thus, their detection in breadfruit and soursop in this study is desirable.

The presence of sterols as observed in African mango (pulp; seeds) and breadfruit is in accordance with a report by Amarasinghe *et al*. (2008). It was not detected in soursop

contrary to a report by Adewole and Ojewole (2008) as well as not detected in sweetsop. Awad and Fink (2000) suggested strong inverse relations between intake of plant sterol and risk of some cancers. This was corroborated by De Stafani *et al.* (2000) in a controlled case-study that showed the consumption of plant sterols to be inversely proportional to the occurrence of stomach cancer. The detection of this plant chemical in breadfruit and African mango (pulp and seeds) therefore, suggests the potential health benefits associated with the consumption of the fruits.

The general test for triterpenoids gave positive inferences for all the samples except for soursop. The detection of the chemical in breadfruit and African mango seeds has been reported by Wang *et al*. (2006) and Wolfe *et al*. (2010) but data on its presence in African mango pulp and sweetsop is scarce although it was detected in this study. Sweetsop which tested positive to triterpenoids, had a negative inference for the sterol test. This corroborates an assertion by Trease and Evans (2002) that all sterols are triterpenoids but the vice-versa is untrue. Varadharajan *et al*. (2012) reported wound healing properties responsible for wound contraction and epithelialisation by triterpenoids – a potential that makes their presence in foods desirable. Thus, the detection of triterpenoids in the samples further indicates the medicinal potential of the edible portions of the studied fruits.

### 4.4 Antioxidant activity (AOA) of fruit samples

The studied fruits showed high free radical scavenging (antioxidant) activities against DPPH compared to the activity of the synthetic antioxidant, BHT (91.76±0.11%), with their activities ranging from 60.56±1.63% (African mango seeds) to 75.45±0.49% (African mango pulp). The results are presented in Fig 4.2.

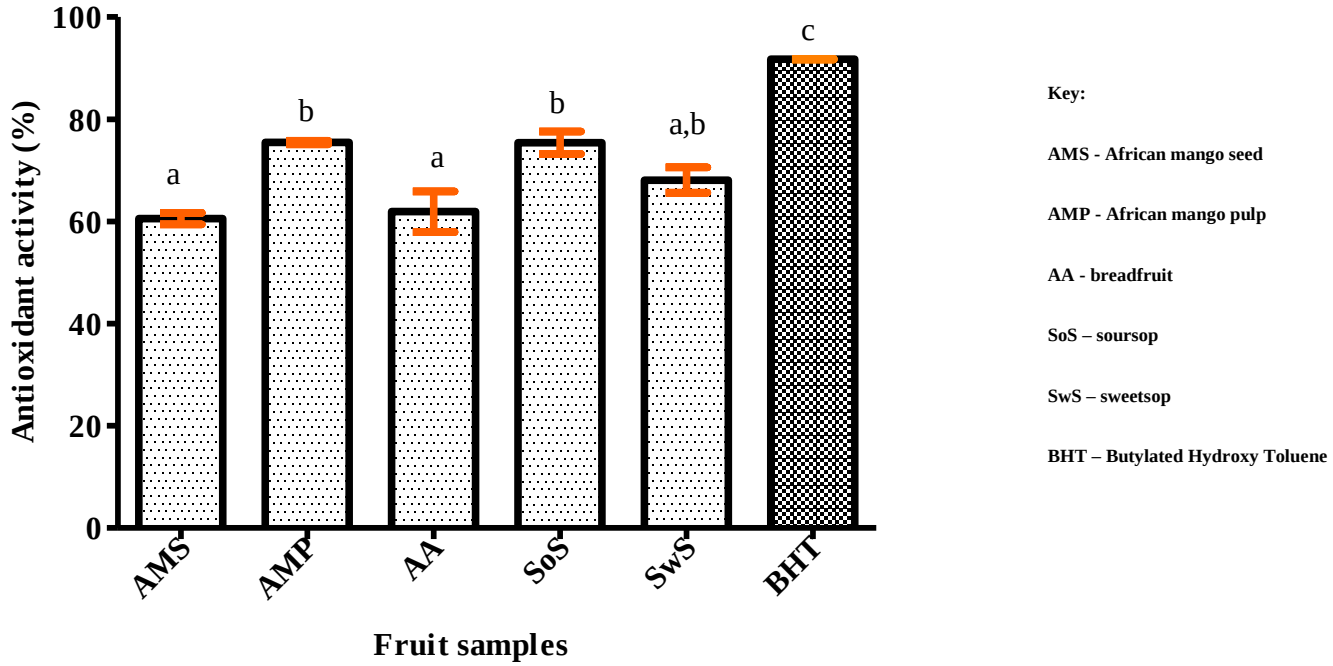



Fig 4.2: Free-radical scavenging activity of fruit samples against DPPH

Plots with different alphabets indicated on top are statistically different ($P < 0.05$)

Kolar *et al*. (2011) reported an activity of 59.29% (against DPPH) for an Indian variety of breadfruit. Similarly, Murillo *et al*. (2012) reported relatively high scavenging activity against DPPH for some Panama varieties of fruit pulp of soursop, while Abgor *et al*. (2011) also reported a high scavenging activity against DPPH for African mango seeds in varieties from Cameroun. Though not ample for objective comparison, these reports corroborate the fairly high scavenging activities (against DPPH) obtained for the Ghanaian varieties of soursop (75.39±3.10%), breadfruit (61.93±5.61%) and African mango seeds (60.56±1.63%) in this study. This study further reports a high AOA for African mango pulp and sweetsop with radical scavenging activities of 75.45±0.49% and 68.10±3.47%, respectively.

The obtained AOA of the underutilised fruits also compared favourably with that of apple pulp (an acclaimed fruit with good AOA) with an activity of 69.1% (Leontowicz *et al*., 2003) but were superior in terms of their AOA to some other common fruits viz., banana with

activities ranging from 26.55% - 29.38% for two varieties (Fatemeh *et al*., 2012) and orange with 47.5% - 49.2% (Klimczak *et al*., 2006). In addition to dietary fibre, Murillo *et al*. (2012) and Rawat *et al*. (2010) proposed the AOA as an important parameter in measuring the health and nutritional potential of fruits. A review by Liu (2003) also confirmed this and suggested the different mechanisms of antioxidants (prominently, scavenging free radicals) to be responsible for the inverse relations between their consumption and many non-communicable diseases including coronary heart diseases, diabetes and some cancers. Thus, the fairly high free-radical scavenging capacity (AOA) of the edible portions of the studied underutilised fruits (compared with the common fruits on the market) is much needed in seeking optimum health of the populace.

### 4.4.1 Ascorbic acid (vitamin C) content of samples

The analysis of all the fruit mesocarps showed considerable but varying quantities of ascorbic acid with values ranging from 20.33±0.17 mg/100 g for sweetsop to 63.67±0.05 mg/100 g for soursop (Fig 4.3). The only seed sample in this study, African mango seeds, had a low ascorbic acid content of 1.00±0.03 mg/100 g.

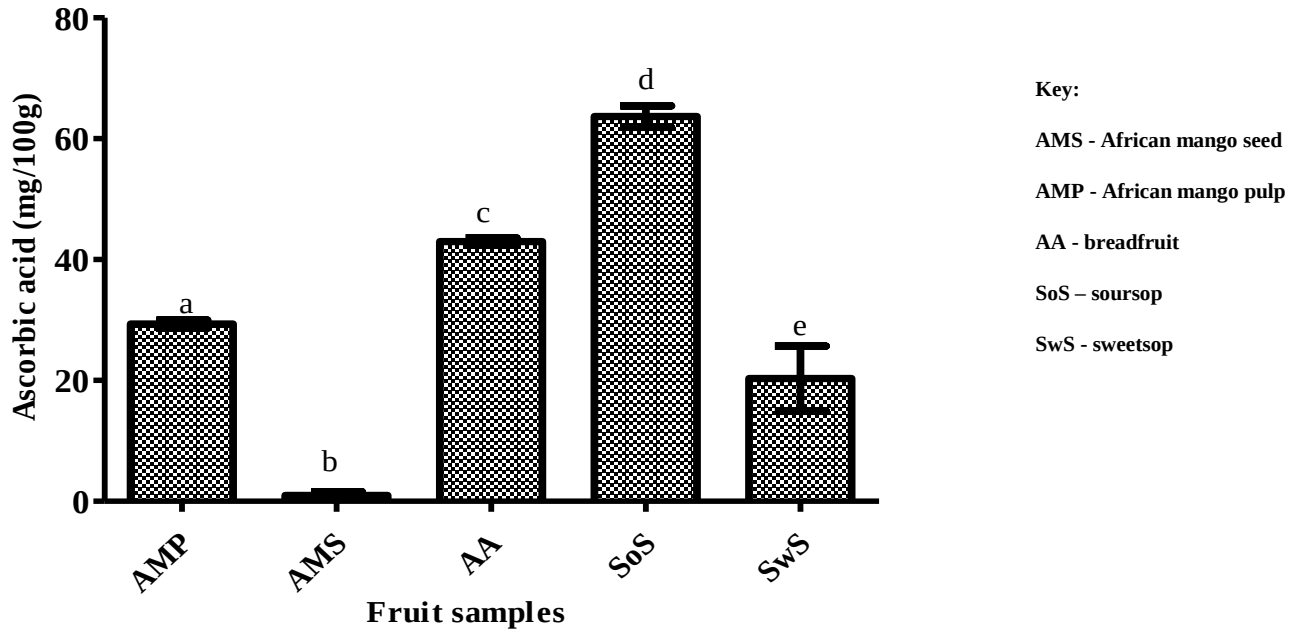



Fig 4.3: The ascorbic acid content of edible portions of four underutilised fruits

Plots with different alphabets indicated on top are statistically different ($P < 0.05$)

The ascorbic acid content of soursop was high – more than double the value, 21.83±3.99 mg/100 g, reported by Vera de Rosso (2013) for some Brazilian varieties. Similarly, breadfruit with obtained value of 43.00 mg/100 g was higher than the reported values which ranged from 18.2 – 23.3 mg/100 g for Hawaiian varieties (Meilleur *et al*., 2004). On the other hand, values for sweetsop (20.33 mg/100g) and African mango pulp (29.33 mg/ 100 g) were relatively lower than the reported values by Pinto *et al*. (2005) (30 mg/ 100 g) and Okiei *et al*. (2009) (36.13 mg/ 100 g), respectively. According to Lee and Kadar (2000), vitamin C contents of fruits are influenced by a number of factors and prominent among them include varietal differences and pre-harvest environmental conditions. Any of these factors may have contributed to the variations in the ascorbic acid contents of the samples from that of the stated reports.

Vitamin C in foods has been associated with antioxidant activity and therapeutic effects including maintenance and protection of skin and teeth as well as the prevention of scurvy (Shofian *et al*., 2011). However, vitamin C, as reported by Okiei *et al*. (2009) and Ismail and Fun (2003) must be supplied daily with a recommended allowance of 60 mg. Although Tosun and Yücecan (2007) suggested it is present in appreciable quantities in other food sources such as green vegetables and potatoes, Kadar (2002) reported it is easily deactivated by heat and exposure to the atmosphere, because of its strong reducing properties. Thus, their presence in fruits and vegetables that do not require heating is preferred.

Moreover, compared to the ascorbic acid content reported by Lee and Kadar (2002) for some readily available fruits, banana (15.3 mg/100g) and watermelon (8.0 mg/100g), the values obtained for the studied underutilised fruits suggest the potential of these fruits to significantly complement the daily vitamin C requirements of consumers when incorporated into diets as raw-eaten snacks. The ascorbic acid content of breadfruit and soursop is comparable to the ascorbate composition of fruits acclaimed as high vitamin C sources such as orange and strawberry with reported values of 54.7 mg/100g; 63.33 mg/100ml (Florida and Agege varieties) and 60.0 mg/100g respectively (Lee and Kadar, 2000). Thus, the potential use of the studied fruit mesocarps as alternative vitamin C sources to solve the challenges associated with the monotony of the common few ones cannot be overstated.

There was non-significant correlation ($P < 0.05$) between ascorbic acid content of the studied fruits taken as a whole and their AOA (Appendix 5). This may be attributed to the varying degrees of AOA shown by the ascorbic acid content of the different fruit species.

### 4.4.2 Phenolic content of fruit samples

The total phenolic contents of the fruit samples ranged from 3.71 mg GAE/100g for breadfruit to 20.95 mg GAE/100g for African mango seeds (Fig 4.4).

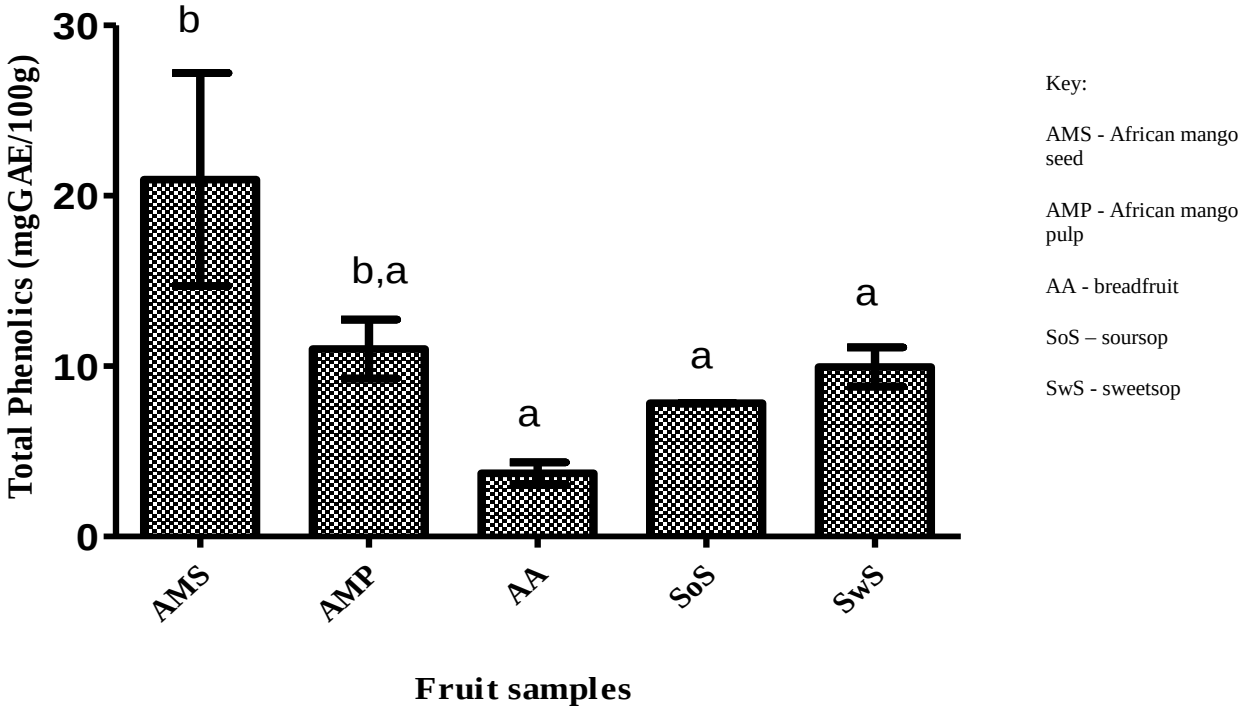



Fig 4.4: The total phenolic contents (mg GAE/ 100 g) of fruit samples

Plots with different alphabets indicated on top are statistically different ($P < 0.05$)

Limited studies on these underutilised fruits viz., Agbor *et al*. (2005) (on African mango seeds from Cameroun), Vera de Rosso (2013) on sweetsop varieties from Brazil and Kolar *et al*. (2011) on some Indian varieties of breadfruit, report of comparatively higher phenolic contents than the obtained values for this study. The variations may be attributed to varietal differences resulting from different climatic and pre-harvest conditions as well as genotypic differences as suggested by Murillo *et al*. (2012). The suggestion has been corroborated by Rawat (2011) who reported significant source specific variations in the phenolic contents of the same species of some fruits.

Deng *et al*. (2012) and Agbor *et al*. (2005) also reported phenolic constituents of plant foods to be either hydrophilic and/ or lipophilic - suggesting their analysis to be invariably influenced by the solvent employed, extraction methods and length of the extraction. These factors may also have contributed to the observed variations indicating the need for an in-depth study of the nature of the inherent phenolics of plant foods especially that of the studied fruits in-order to adequately quantify them. Inspite of this shortfall, the phenol content of the African mango seed (20.95 mg GAE/100g) is comparable to that of pineapple with phenolic content ranging from 20.20 – 26.20 mg GAE/100g and mango with 20.0 – 40.0 mg GAE/100g of total phenols for different varieties (Nixwell *et al*., 2013).

Plant phenolic compounds are known to be associated with their AOA in addition to having other disease prevention potential such as being antimicrobial, antiviral and anti-inflammatory agents as reported by Oskana *et al*. (2012) and Deng *et al*. (2012). However, taken as a whole, there was non-significant correlation ($P < 0.05$) between the total phenolics of the fruits and their AOA (Appendix 6). This finding supports the suggestion by Murillo *et al.* (2012) that the total phenolics of different species of fruits have different degrees of contributions to their AOA and the relationship observed in one, cannot be generalised for all others.

### 4.5 Summary of Discussion

From the discourse, the study suggests the underutilised fruits, especially the *Annona species* and African mango seeds, have fairly high fat and protein contents. They may thus be incorporated into diets as cheaper and/or more accessible source of nutrients to curtail some nutritional deficiencies. Moreover, the relatively high antioxidant activities observed in the selected fruits coupled with the array of phytochemicals detected indicate the potential health benefits of the fruits. This is especially so for sweetsop pulp in that contrary to myths of its

negative impart to health due to its sweetness, this study rather reports the presence of inherent health beneficial constituents, most of which are associated with the treatment of some chronic diseases.

Also, the studied fruits can significantly supplement the dietary fibre needs of the populace (especially that of urban-dwellers) when incorporated into diets as raw-eaten snacks. The fruit mesocarps may also be used as alternative vitamin C sources in solving the challenges associated with the monotony of the traditional commercial ones.

### 4.6 Limitation of study

The soluble solvent extraction method employed in the quantification of total phenols accounted for only the free phenolic compounds and not the bound compounds which are known to cater for about 30% of total phenols (depending on fruit specie).

# CHAPTER FIVE

## 5.0 Conclusion and recommendation

### 5.1 Conclusion

The studied underutilised fruits had appreciable soluble and insoluble dietary fibre fractions with corresponding high total dietary fibre contents viz., 22.7 g/ 100g for African mango pulp, 21.6 g/ 100g for soursop, 18.3 g/ 100g for African mango seeds, 11.7 g/ 100g for breadfruit and 11.5 g/ 100 g for sweetsop. They also showed considerable moisture, protein, ash and carbohydrate contents with values ranging from (40 to 84.07) %, (2.63 to 6.71) %, (2.44 to 4.00) % and (0.65 to 23.24) % respectively.

Generally, the samples showed positive inferences to the major phytochemicals tested except for the presence of alkaloids. This included the detection of coumarins in breadfruit and soursop, cardiac glycosides in soursop, sweetsop and African mango seeds as well as triterpenoids in African mango pulp and sweetsop. The studied underutilised fruits also had fairly high antioxidant (free radical scavenging) activities viz., 75.45% for African mango pulp, 75.39% for soursop, 68.10% for sweetsop, 61.93% for breadfruit and 60.56% for African mango seeds.

Thus, there are potential health beneficial constituents to be derived from the incorporation of these fruits into diets and this indicates the need for their exploitation in seeking optimum health of the populace.

### 5.2 Recommendation

From the findings of this study, it is recommended that, in-depth quantitative studies of the detected phytochemicals especially that of the phenolic compounds be done on the fresh fruits to further evaluate their associated health benefits.

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# APPENDIX

## Appendix 1: ANOVA for Proximate determinations of samples

KEY: AA- breadfruit; IP- African mango pulp; IS- African mango seeds; SOS- soursop; SWS- sweetsop

* denotes a statistically significant difference.

### Moisture

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 3757.72 | 4 | 939.431 | 316.40 | 0.0000 |
| Within groups | 29.6914 | 10 | 2.96914 | | |
| Total (Corr.) | 3787.41 | 14 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AA - IP | * | -16.1777 | 3.13482 |
| AA - IS | * | 27.6918 | 3.13482 |
| AA - SOS | * | -14.4775 | 3.13482 |
| AA - SWS | * | -5.29848 | 3.13482 |
| IP - IS | * | 43.8695 | 3.13482 |
| IP - SOS | | 1.7002 | 3.13482 |
| IP - SWS | * | 10.8792 | 3.13482 |
| IS - SOS | * | -42.1693 | 3.13482 |
| IS - SWS | * | -32.9903 | 3.13482 |
| SOS - SWS | * | 9.17901 | 3.13482 |

### Ash

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 4.34897 | 4 | 1.08724 | 18.81 | 0.0001 |
| Within groups | 0.577886 | 10 | 0.0577886 | | |
| Total (Corr.) | 4.92686 | 14 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AA - IP | | -0.33344 | 0.437339 |
| AA - IS | | 0.199905 | 0.437339 |
| AA - SOS | * | -1.36209 | 0.437339 |

| | | | |
|---|---|---|---|
| AA - SWS | | -0.429175 | 0.437339 |
| IP - IS | * | 0.533345 | 0.437339 |
| IP - SOS | * | -1.02865 | 0.437339 |
| IP - SWS | | -0.0957346 | 0.437339 |
| IS - SOS | * | -1.562 | 0.437339 |
| IS - SWS | * | -0.62908 | 0.437339 |
| SOS - SWS | * | 0.932917 | 0.437339 |

**Protein**

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 34.3019 | 4 | 8.57549 | 215.47 | 0.0000 |
| Within groups | 0.397981 | 10 | 0.0397981 | | |
| Total (Corr.) | 34.6999 | 14 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AA - IP | | 0.239056 | 0.362935 |
| AA - IS | * | -3.84379 | 0.362935 |
| AA - SOS | * | -1.74636 | 0.362935 |
| AA - SWS | * | -0.395101 | 0.362935 |
| IP - IS | * | -4.08284 | 0.362935 |
| IP - SOS | * | -1.98541 | 0.362935 |
| IP - SWS | * | -0.634157 | 0.362935 |
| IS - SOS | * | 2.09743 | 0.362935 |
| IS - SWS | * | 3.44869 | 0.362935 |
| SOS - SWS | * | 1.35126 | 0.362935 |

**Fibre**

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 237.012 | 4 | 59.2531 | 60.18 | 0.0000 |
| Within groups | 9.84625 | 10 | 0.984625 | | |
| Total (Corr.) | 246.859 | 14 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AA - IP | * | -2.06476 | 1.80523 |
| AA - IS | * | -11.4214 | 1.80523 |
| AA - SOS | * | -2.72392 | 1.80523 |
| AA - SWS | * | -2.2621 | 1.80523 |
| IP - IS | * | -9.35664 | 1.80523 |
| IP - SOS | | -0.659167 | 1.80523 |
| IP - SWS | | -0.197343 | 1.80523 |

| | | | |
|---|---|---|---|
| IS - SOS | * | 8.69747 | 1.80523 |
| IS - SWS | * | 9.1593 | 1.80523 |
| SOS - SWS | | 0.461825 | 1.80523 |

**Fat**

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 282.935 | 4 | 70.7338 | 618.13 | 0.0000 |
| Within groups | 1.14431 | 10 | 0.114431 | | |
| Total (Corr.) | 284.08 | 14 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AA - IP | | -0.249095 | 0.615418 |
| AA - IS | * | -11.3032 | 0.615418 |
| AA - SOS | * | -0.958073 | 0.615418 |
| AA - SWS | * | -0.704548 | 0.615418 |
| IP - IS | * | -11.0541 | 0.615418 |
| IP - SOS | * | -0.708978 | 0.615418 |
| IP - SWS | | -0.455453 | 0.615418 |
| IS - SOS | * | 10.3451 | 0.615418 |
| IS - SWS | * | 10.5986 | 0.615418 |
| SOS - SWS | | 0.253525 | 0.615418 |

**Carbohydrates**

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 1285.8 | 4 | 321.449 | 54.89 | 0.0000 |
| Within groups | 58.5645 | 10 | 5.85645 | | |
| Total (Corr.) | 1344.36 | 14 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AA - IP | * | 18.5859 | 4.40266 |
| AA - IS | | -1.32335 | 4.40266 |
| AA - SOS | * | 21.2679 | 4.40266 |
| AA - SWS | * | 9.08941 | 4.40266 |
| IP - IS | * | -19.9093 | 4.40266 |
| IP - SOS | | 2.68201 | 4.40266 |
| IP - SWS | * | -9.49652 | 4.40266 |
| IS - SOS | * | 22.5913 | 4.40266 |
| IS - SWS | * | 10.4128 | 4.40266 |
| SOS - SWS | * | -12.1785 | 4.40266 |

**Appendix 2: ANOVA for antioxidant activity of samples**

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 1322.97 | 5 | 264.594 | 28.29 | 0.0004 |
| Within groups | 56.1194 | 6 | 9.35324 | | |
| Total (Corr.) | 1379.09 | 11 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AMS - AMP | * | -14.8979 | 7.48343 |
| AMS - AA | | -1.36867 | 7.48343 |
| AMS - SOS | * | -14.8344 | 7.48343 |
| AMS- SWS | * | -7.54015 | 7.48343 |
| AMS - BHT | * | -31.207 | 7.48343 |
| AMP - AA | * | 13.5292 | 7.48343 |
| AMP- SOS | | 0.0634786 | 7.48343 |
| AMP - SWS | | 7.35774 | 7.48343 |
| AMP - BHT | * | -16.3092 | 7.48343 |
| AA - SOS | * | -13.4657 | 7.48343 |
| AA - SWS | | -6.17148 | 7.48343 |
| AA - BHT | * | -29.8384 | 7.48343 |
| SOS - SWS | | 7.29426 | 7.48343 |
| SOS - BHT | * | -16.3726 | 7.48343 |
| SWS - BHT | * | -23.6669 | 7.48343 |

**Appendix 3: ANOVA for phenolic compounds of samples**

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 324.945 | 4 | 81.2363 | 4.65 | 0.0615 |
| Within groups | 87.4301 | 5 | 17.486 | | |
| Total (Corr.) | 412.375 | 9 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AMP - AMS | | -9.93 | 10.7492 |
| AMP - AA | | 7.2905 | 10.7492 |
| AMP - SOS | | 3.18 | 10.7492 |
| AMP - SWS | | 1.055 | 10.7492 |
| AMS - AA | * | 17.2205 | 10.7492 |
| AMS - SOS | * | 13.11 | 10.7492 |
| AMS - SWS | * | 10.985 | 10.7492 |
| AA - SOS | | -4.1105 | 10.7492 |
| AA - SWS | | -6.2355 | 10.7492 |
| SOS - SWS | | -2.125 | 10.7492 |

**Appendix 4: ANOVA for vitamin C**

| *Source* | *Sum of Squares* | *Df* | *Mean Square* | *F-Ratio* | *P-Value* |
|---|---|---|---|---|---|
| Between groups | 6679.73 | 4 | 1669.93 | 84.34 | 0.0000 |
| Within groups | 198.0 | 10 | 19.8 | | |
| Total (Corr.) | 6877.73 | 14 | | | |

| *Contrast* | *Sig.* | *Difference* | *+/- Limits* |
|---|---|---|---|
| AA - AMP | * | 13.6667 | 8.09525 |
| AA - AMS | * | 42.0 | 8.09525 |
| AA - SOS | * | -20.6667 | 8.09525 |
| AA - SWS | * | 22.6667 | 8.09525 |
| AMP - AMS | * | 28.3333 | 8.09525 |
| AMP - SOS | * | -34.3333 | 8.09525 |
| AMP - SWS | * | 9.0 | 8.09525 |
| AMS - SOS | * | -62.6667 | 8.09525 |
| AMS - SWS | * | -19.3333 | 8.09525 |
| SOS - SWS | * | 43.3333 | 8.09525 |

**Appendix 5: Correlation between antioxidant activity and vitamin C (ascorbic acid) of samples**

| | | Vitamin C | Antioxidant Activity |
|---|---|---|---|
| Vitamin C | Pearson Correlation | 1 | .565 |
| | Sig. (2-tailed) | | .321 |
| | N | 5 | 5 |
| Antioxidant Activity | Pearson Correlation | .565 | 1 |
| | Sig. (2-tailed) | .321 | |
| | N | 5 | 5 |

**Appendix 6: Correlation between antioxidant activity and total phenolics of samples**

| | | Total phenols | Antioxidant Activity |
|---|---|---|---|
| Total phenols | Pearson Correlation | 1 | -.292 |
| | Sig. (2-tailed) | | .634 |
| | N | 5 | 5 |
| Antioxidant Activity | Pearson Correlation | -.292 | 1 |
| | Sig. (2-tailed) | .634 | |
| | N | 5 | 5 |

**Appendix 7: Coefficients for correlations in Appendices "5" and "6"**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 39.159 | 51.237 | | .764 | .525 |
| | Vitamin C | .273 | .276 | .899 | .986 | .428 |
| | Total phenols | 64.174 | 138.814 | .421 | .462 | .689 |

Dependent Variable: Antioxidant Activity