Quantification and Inter-Relationship between Microbial Load, Disease, Proximate Composition and Phytochemical Content of Postharvest *Irvingia* Fruit Waste

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Abstract
Irvingia species are important fruit trees. In this study, microbial load, disease, proximate and phytochemical components of postharvest Irvingia fruit wastes quantified, and their inter-relationship studied. Results showed that postharvest days after harvest (DAH) significantly influenced weight, bacterial and fungal populations, proximate and phytochemical contents of Irvingia fruit wastes. Cumulative mean weight and disease severity of the fruits were 42.6g and 31.35% respectively. Mean population of bacteria and fungi per gram of fruit were 1.02 × 10^7 and 7.08 × 10^5 Cfu, respectively. Mean moisture content was 83.37% while mean proximate and phytochemical contents per 100g of fruit waste were protein (0.64g), lipid (0.12g), Fibre (0.74g), carbohydrate (14.30g), alkaloids (4.86g), tannins (3.83g), saponins (1.90g) and flavonoids (4.25g). Correlation- regression analyses showed that all the parameters assessed were inter-related. Specifically, alkaloids and tannins were separately significantly (P≤0.05) related to fruit weight, disease, bacterial and fungal populations, moisture, lipid, fibre and carbohydrate. Whilst carbohydrate was significantly (P≤0.05),
directly related to all phytochemicals, lipid was inversely related to all phytochemicals except flavonoids. The balance of proximate and phytochemical contents is apparently governed by the metabolic need of Irvingia fruit vis-à- vis the prevailing threat of pathogens and abiotic factors. Investigating the biotic and abiotic factors that influence the biosynthetic processes of proximate and phytochemical contents in the fruits to maintain the right mix/balance for human and animal consumption is herein recommended.

Keywords: *Irvingia, proximate composition, phytochemicals, bacterial and fungal CFU*

INTRODUCTION

Irvingia species are economically important, high-value indigenous multi-purpose tree species found in the wild forests of most West and Central African countries (Harris, 1996; Lowe *et al.*, 2000). The fruits are ellipsoidal, mango-shaped drupe; measuring 4-7cm long with the pericarp differentiated into exocarp which forms the peel, and mesocarp, the fleshy pulp and the endocarp which is the hardstone enclosing the kernel (Harris, 1996; Etebu, 2013). Until very recently, most studies have been centered on the kernels which are rich in fat, oil and protein (Onimawo and Egbekun, 1998; Ihekoronye and Ngoddy, 1985).

Locals generally harvest the fruits from the wild to extract the kernels which is primarily used as a thickening condiment in sauce preparation (Matos *et al.*, 2009) while the fleshy part which remarkably constitute over 80% of the whole fruit are treated as waste; thrown away to rot in dumps, water bodies, pits or nearby bushes (Etebu, 2012; Ladipo *et al.*, 1996).

Interestingly, a few reports have highlighted the potentials of processing the fleshy pulp of *Irvingia* fruits into fruit drinks, wine, jam and other syrups, as well as feed for pigs (Ayuk, 1999; Leakey *et al.*, 2003; Okafor, 1985; Shiembo *et al.*, 1996). These sketchy reports have motivated some researchers to separately assess the proximate composition (Onimawo *et al.*, 2003; Etebu and Tungbulu, 2016), vitamins (Etebu, et al., 2016) and phytochemical content (Tungbulu, et al., 2016) of the fleshy pericarp of the fruit. Owing to the high susceptibility of postharvest *Irvingia* fruits to disease, the potential relationship between these components and brownish-black rot disease were separately studied in these works.

Although these isolated studies have shown the nutritional endowment of what was hitherto considered a waste, conducting a single comprehensive study is imperative to give a clear holistic perspective involving the fungal and bacterial load, disease, proximate and phytochemical content of postharvest *Irvingia* fruit wastes. Furthermore, it is also imperative to study the potential relationships between proximate contents and phytochemicals of *Irvingia* fruits because most phytochemicals have been shown to occur in plants as conjugates with sugars, fatty acids, or proteins (Shahidi and Wanasundara, 1997; Wyen *et al*., 2000). Hence in this research brownish-black rot disease, microbial load, proximate composition and phytochemical content was quantified, and their potential interrelationships were studied. Findings from this work would enable us have a holistic view regarding the nutritional and health potentials of postharvest *Irvingia* fruits that could be harnessed to add value to the well-being of human society.

Materials and Methods

Samples collection and Experimental design

Irvingia fruits were harvested from a natural forest situated in Toru-Angiama (Lat. 5° 7′N Long. 6° 6′ E) of Bayelsa state, Nigeria. The experiment was designed according to Etebu, 2012, 2013, Etebu and Tungbulu, 2015, Etebu *et al.*, 2016; Tungbulu et al., 2016 with slight modifications. Briefly, a total of 30 fresh and green *Irvingia* fruits were randomly selected and split with a machete to extract the kernel, and the pericarp which is usually considered waste by locals were thereafter separated into three replicates (300 fruits per replicate). A quadrant measuring about 3m × 1m having three equal compartments (representing three replicates) of 1m x 1m was constructed and the fruit wastes from each replicate were separately spread into the three compartments of the quadrant. The quadrant was barricaded at the sides with nets to exclude reptiles and the fruits were left to decay for 6 Days after harvest (DAH).

Weight and disease assessment

At the onset (representing DAH 0) of the experiment 5 fruit wastes were randomly selected each from all three replicates and their weights and postharvest disease severity were individually assessed based on brownish-black rot disease symptom described by Etebu (2012). Assessment of postharvest disease of the fruits was repeated on the 3rd and 6th days after harvest (DAH) respectively. Severity of postharvest spoilage was determined visually by the proportion of fruit area affected by brownish-black rot disease and expressed in percentage as according to Etebu *et al.* (2003, 2009).

Preparation of *Irvingia* fruit waste slurry

The same 5 fruit wastes assessed for disease and weight on each of the respective DAH (0, 3 and 6 days) were separately surface sterilized in 0.75% Sodium hypochlorite for 10mins and rinsed thoroughly in sterile tap water. Thereafter, the fleshy part (exocarp and mesocarp) of

the fruit wastes were sliced and blended with a household blender for 30s under aseptic conditions to form *Irvingia* fruit waste slurry.

Determination of microbial population

One (1) ml of *Irvingia* fruit slurry was serially diluted and plated onto Nutrient agar (Oxoid Ltd, Hampshire, UK) previously impregnated with a fungicide, Itraconazole (4µgml-1) according to Cutsem (1989) to assess the bacterial population.

Also, the same serially diluted *Irvingia* fruit slurry was plated in 3 replicates onto Sabouraud dextrose agar (Oxoid Ltd, Hampshire, UK) previously prepared according to manufacturer's prescription, and integrated with 50µg ml^{-1} each of streptomycin and tetracycline according to Etebu *et al*. (2003) to assess fungal population.

The Nutrient agar plates were incubated at 28°C for 2 days while the Sabouraud dextrose agar plates were incubated at ambient room temperature for 3 days. Bacterial Colony Forming Units per gram of fruits on Nutrient agar were assessed after 2 days of incubation, while fungal Colony Forming Units on Sabouraud dextrose agar were assessed after 3 days of incubation.

Determination of proximate composition

Different parameters constituting proximate content of *Irvingia* fruit wastes slurry were determined using standard procedures as described by Etebu and Tungbulu (2016a). Standard procedures were carried out as follows, moisture (AOAC, 2000), fibre (AOAC, 1986), protein (AOAC, 1986; Kirk and Sawyer 1991), lipids (Osborne and Voogt, 1978; AOAC, 1986), carbohydrate (Eyeson and Ankrah, 1975)

Phytochemical content determination

One hundred (100) gram of the resultant slurry, produced as described above, was treated in various ways to screen for the different types of phytochemicals according to Harbone (1973), Sofowora (1993) and Trease and Evans (1989).

Thereafter, the different groups of phytochemicals (alkaloids, flavonoids, saponins and tannins) were quantified as described by Tungbulu *et al* (2016), using standard procedures according to Harborne, (1973), Zhishen *et al.*, (1999) and Swain (1979). The different phytochemicals were determined in grams per 100g of fresh *Irvingia* fleshy fruit waste.

Data analysis: Brownish-black rot disease assessments were arcsine transformed according to Gomez and Gomez (1984). Also, Percentage moisture data were square root transformed and data on colony forming units of bacteria and fungi were separately logarithm transformed prior to statistical analyses. The transformed data alongside other data were subjected to ANOVA using Generalized Linear Model of SPSS version 16.0 Statistical software, and the resulting means were further subjected to Tukey's mean separation. Correlation/regression analyses were also performed between all parameters using Minitab Statistical software version 14.0. Means of transformed data were thereafter de-transformed (weighted) and discussed hereunder alongside other parameters. Comparison of weight, disease, proximate and phytochemical contents were made with respect to days after harvest, and the inter-relationships between all parameters were discussed.

Results and discussions

Brownish-black rot disease

The response of *Irvingia* fruit wastes left to rot under open field conditions after harvest was characterized by a ripening process and brownish-black rot disease (data not shown) as previously described in several earlier reports (Joseph and Aworh, 1991, 1992; Etebu, 2012, 2013, Etebu and Tungbulu, 2016). Ripening process of climacteric fruits are subject to physiological and metabolic changes that render them vulnerable to microbial attack (Prusky and Keen, 1993). Several microorganisms have been shown to associate with diseased postharvest *Irvingia* fruits (Joseph and Aworh, 1992; Etebu, 2012, 2013; Etebu and Tungbulu, 2015). However, only the fungus *Botrytis* species has been reportedly proven to be the primary causal agent of brownish-black rot disease of postharvest Irvingia fruits (Joseph and Aworh, 1992).

Brownish black rot disease of postharvest *Irvingia* fruits was significantly ($P \leq 0.05$) influenced by postharvest period described in this work as days after harvest (DAH) (Table 1). The effect of DAH on brownish-black rot disease of the fruits were comparable to recent findings of several earlier works (Etebu and Tungbulu, 2015, Etebu *et al*., 2016; Tungbulu *et al*., 2016) Severity of postharvest disease was observed to be dependent on the number of days after harvest (DAH). Disease progressed significantly ($P \leq 0.05$) as DAH increased (Table 1). Mean weighted brownish-black rot disease scores on 0^{th}, 3^{rd} and 6^{th} DAH in this work were 4.35%, 23.68%, and 76.48%, respectively. Although these values followed the same pattern of disease severity increment with the passage of postharvest period, they were nonetheless different from the results of some of the earlier works. For example, Etebu *et al* (2016) reported that disease severity of *Irvingia* fruits was as much as 87.75% after 6 days of harvest, as compared to 76.48% observed in this present work. Differences observed in severity of brownish black rot disease of postharvest *Irvingia* fruits between this work and that of Etebu *et al*., (2016) could be due to potential variability in the individual fruit samples, occasioned by differences in growth conditions of the trees and differences in environmental conditions not captured during experimentation.

Fruit waste weight

In contrast to brownish black rot disease, weight of *Irvingia* fruits progressively reduced as DAH increased (Table 1). De-transformed weights of fruit wastes at 0^{th}, 3^{rd} and 6^{th} DAH in this work were 51.55g, 42.64g and 34.57g respectively, each being significantly ($P \leq 0.05$) different from the other. These results were different from the findings of a similar work done by Etebu and Tungbulu (2015). Firstly, the fruit wastes in this present work were clearly heavier than those reported by Etebu and Tungbulu (2015). Secondly, whilst the difference in weight of the fruits at DAH 0 and 3 were not significant in this earlier report, the findings from this present work showed a significant difference between same treatments. The difference in the weight of *Irvingia* fruit wastes could have been as a result of the different locations from which the fruits were harvested. Whilst *Irvingia* fruits were harvested from a wild forest in Amassoma (Lat. 4°58′09″N Long. 6°06′34″ E), Bayelsa State, Nigeria in the former work (Etebu and Tungbulu, 2015), the fruits used in this present work were harvested from a wild forest in Toru-Angiama (Lat. 5° 7′N Long. 6° 6′ E) also in Bayelsa State, Nigeria. Variability in crops is known to be influenced by genotypic and phenotypic factors (Turner *et al*., 1990), which could be occasioned by soil conditions amongst other factors.

Microbial population

Bacterial and fungal populations associated with postharvest *Irvingia* fruit wastes were also observed to be dependent on the postharvest period defined by DAH in this work (Table 1). Weighted Bacterial count observed to be associated with postharvest *Irvingia* fruits on the 0^{th}, 3^{rd} and 6^{th} DAH was 7.24E+06, 1.29E+07, and 1.04+07 per gram, respectively. Bacterial count on the fresh fruits (DAH=0) were significantly lower than those counted on the fruits on the 3^{rd} and 6^{th} DAH. Bacterial counts on fruits on the 3rd and 6^{th} day after harvest were not significant at the 5% probability level. Research works showing bacterial population of postharvest *Irvingia* fruits fleshy pericarp are rare. Recent work by Etebu and Tungbulu (2015) on the bacterial quality of *Irvingia* fruits was qualitative, no attempt was made to quantify the bacterial population. Whilst, bacteria were recovered from postharvest *Irvingia* fruit pulp up to the 6^{th} day after harvest in this work (Table 1), Etebu and Tungbulu (2015) indicated that bacteria could not be recovered from the fruits after 3 days of harvest. In attempting to explain the non-recovery of bacteria from *Irvingia* fruits left to rot on soil after 6 days, Etebu and Tungbulu (2015) posited that decaying *Irvingia* fruits may have produced chemical substances that inhibited bacterial growth. Secondly, they also hypothesized that the lack of growth of bacteria on the fruits as from the 6^{th} DAH could have been due to chemical substances produced by fungi or endospore forming bacteria. Results in this present work, however, showed that bacteria are able to persist in postharvest *Irvingia* fruits beyond the 3^{rd} day after harvest. Although the bacterial community structure was not studied in this work, recent bioinformatics analyses of partial 16S rRNA gene sequences amplified from bacterial isolates recovered from postharvest *Irvingia* fruit pulp shared ≥ 99% DNA sequence similarity with *Bacillus* spp., *Enterobacter* spp., *Oceanobacillus profundus*, and *Staphylococcus cohnii* (Etebu and Tungbulu, 2015).

Fungal population assessed in this work was relatively lower than the bacterial population irrespective of postharvest period. Also, in contrast to bacterial population, results showed that the fungal population increased significantly ($P \leq 0.05$), progressively as DAH increased. Fungal count at 0^{th}, 3^{rd} and 6^{th} DAH was 4.57E+05, 8.31E+05 and 1.05E+06 cfu per gram respectively (Table 1). A relatively recent work showed that, apart from postharvest period, fungal population is significantly influenced by *Irvingia* species (Etebu, 2013). In particular, *I. wombolu* (the bitter species) was reported to sustain a significantly lower fungal population than *I. gabonensis* (the sweet species). Weighted mean fungal population in this present work was 7.79E+05 Cfu per gram of fruit, substantially lower than findings of Etebu (2013).

Earlier works have altogether shown that fungal species belonging to five genera - *Aspergillus, Penicillium, Botrytis, Rhizopus* and *Mucor* are associated with decaying *Irvingia* fruits (Etebu, 2012, 2013; Joseph and Aworh, 1992).

Proximate composition

The amount of the different components of proximate contents of *Irvingia* fruit wastes were varied and were significantly ($P \leq 0.05$) dependent on DAH (Table 1). The proximate composition of food has been variously reported to include its content of protein, carbohydrates, lipids, moisture and dietary fibre (Onimawo and Egbekun, 1998; Ihekoronye and Ngoddy, 1985). Results showed that cumulative weighted mean moisture content of postharvest *Irvingia* fruit wastes was 83.37% while means of other proximate contents per 100g of fruit waste were carbohydrate (14.30g), fibre (0.74g), protein (0.64g) and lipid (0.12g) (Table 1). These results were comparable to results obtained from a similar experiment recently conducted by Etebu and Tungbulu (2016) but varied slightly from

findings of Onimawo and associates (2003) published over a decade ago, and were observed to be widely at variance with findings of Boakye *et al*., (2014).

Although the different proximate constituents of postharvest *Irvingia* fruits assessed in this work were observed to be significantly influenced by postharvest period, the extent of influence varied amongst the different proximate constituents assessed.

Moisture: *Irvingia* fruit wastes were observed to have as much as 83.25% of its weight attributable to Moisture (Table 1). The amount of moisture was significantly dependent on the postharvest period; diminished significantly as days after harvest, increased. A few earlier workers had reported similar findings (Onimawo *et al.,* 2003; Etebu and Tungbulu, 2016). *Irvingia* fruit, like other drupes, turns succulent when ripe; as such the high moisture content of *Irvingia* fruits was expected. The water content of *Irvingia* fruits has been reported to be comparable to that of mango (*Mangifera indica*) which has been shown to constitute 80% water (Mamiro *et al.* 2007; Etebu and Tungbulu, 2015). Furthermore, the sugar concentration of *Irvingia* juice has been reported to be comparable to those of pineapple and orange; having an extraction rate of as much as 75% (Akubor, 1996). Like mango, pineaaple and orange, these comparable attributes of *Irvingia* fruit pulp could be exploited in Nigeria to produce Irvingia juice and jam at commercial rates.

Carbohydrate: Mean carbohydrate concentration across all postharvest period was 14.30g per 100g of postharvest *Irvingia* fruit wastes (Table 1). Like Moisture, postharvest period significantly affected carbohydrate content, but contrary to moisture, the concentration of carbohydrate increased as postharvest period increased (Table 1). Onimawo *et al.*, (2003) showed that 100g of fresh *Irvingia* fruits (Similar to fruits DAH=0 in this work) contained 10.7g of carbohydrates per 100g) as against 12.08g/100g of fruit observed in this work (Table 1). Whilst the carbohydrate content of fresh *Irvingia* fruit assessed in this work could be said to be comparable to the findings of Onimawo et al., (2003) and, Etebu and Tungbulu (2016), it was obviously different from the findings of Boakye *et al.*, (2014).

Protein: The protein content of 100g of *Irvingia* fruit wastes was 0.65g at DAH (0). This decreased to 0.58g at DAH (6) (Table 1). The protein content on DAH (0) was not significantly ($P=0.05$) higher in comparison to its content on the 3rd DAH, while the protein content of the fruits at DAH 0 and 3 were separately significantly ($P\leq0.05$) higher than those assessed on the 6th day after harvest. Although Etebu and Tungbulu (2016) also showed that protein content of *Irvingia* fruits decreased steadily with time after harvest, the difference in the amount of protein in *Irvingia* fruits at DAH 3 and DAH 6, in contrast to the findings of this work, was not significant at $P=0.05$. The findings of this work showed that *Irvingia* fruits possess a relatively low amount of protein, in tandem with what is generally known about proteins in fruits (Kader and Barret, 2005; Nixwell *et al*., 2013). However, a few workers had indicated that the protein content of *Irvingia* fruits could be as much as 1.09 – 2.63% (Onimawo *et al* 2003; Boakye *et al*., 2014). Although differences in *Irvingia* species, growth conditions of fruit trees, soil conditions and storage conditions prior to assessment are some of the potential reasons adduced for the difference in protein contents of *Irvingia* fruits (Etebu and Tungbulu, 2016), there is a need to thoroughly study the factors that could account for the variability in protein content of *Irvingia* fruits. This is because variability in biochemical composition significantly limits the industrial use of crops (Guéguen, 1991), and more importantly, the nutritional value of any given food is defined by its protein content and protein energy (Gopalan *et al*., 2000).

Fibre: Results showed that fibre content per 100g of *Irvingia* fruit pulp decreased from 0.83g on DAH (0) to 0.78g and 0.62g on the 3rd and 6th DAH respectively. In contrast to findings by Etebu and Tungbulu (2015) fibre content of *Irvingia* fruit on DAH (0) decreased progressively, significantly (*P=0.05*) as days after harvest increased. The potential benefits of dietary fibre of *Irvingia* fruit have been discussed in earlier works (Etebu and Tungbulu, 2015; Ngodi *et al*., 2005).

Whilst the results of this work could be said to be comparable to results obtained from the pioneering work of Onimawo and Associates (2003), and a recent work by Etebu and Tungbulu (2016), notwithstanding the slight variations, they are nonetheless significantly different from results reported by Boakye *et al*., (2014). This latter report showed that 100g of fresh *Irvingia* fruit pulp contained Moisture (84.07%), total fibre (22.70g), protein (2.63g), fat (2.84g) and carbohydrate (3.33g). These results as reported by Boakye et al., (2014) are clearly at variance with both the findings of this present work as well as results obtained from the pioneering works of Onimawo *et al.*, (2003), and Etebu and Tungbulu (2016); particularly with respect to total fibre, protein, fat and carbohydrate. Suffice to say Boakye *et al*., (2014) carried out their work with *Irvingia* fruits obtained from Ghana as against Nigeria which was the case with this work. Differences in proximate parameters of same fruits by different workers is not uncommon, and they could be influenced by many factors ranging from genetic differences of *Irvingia* fruits assessed to differences in growth conditions of the fruit trees, health status of the fruits as at the time of investigation, the storage conditions under which they were stored prior to assessment etc.

Phytochemicals

Results showed that postharvest *Irvingia* fruits are endowed with phytochemicals. Overall mean phytochemical contents per 100 gram of Irvingia waste assessed in this work were alkaloids (4.86g), flavonoids (4.25g), tannins (3.83g), and saponins (1.90g) (Table 1). The presence of phytochemicals in fruits, including *Irvingia*, is widely known and reported by several workers (Schreiner and Huyskens-Keil, 2006; Etebu, 2012, 2013, Tungbulu, *et al.,* 2016). Phytochemicals are known to perform important roles in the adaptation and survival of their host plants; these include deterring herbivores, protecting host plants against pathogens or various abiotic stresses, and serving as antioxidants or signaling molecules (Schreiner and Huyskens-Keil, 2006). Similar to the findings of this work, results of an earlier work on *Irvingia* fruits also showed that alkaloids> flavonoids > tannins > saponins (Tungbulu, *et al.,* 2016).

Results further showed that all the different types of phytochemicals were separately, significantly (*P≤0.05*) influenced by postharvest period (Table 1). In general, all the phytochemicals increased significantly (*P≤0.05*) as DAH increased from 0 to 3 (Table 1). Apart from alkaloids, the difference in the amount of phytochemicals in fruits on the 3rd and 6th DAH were not significant at *P=0.05*. Results on phytochemical contents of postharvest *Irvingia* fruit wastes assessed in this work were similar to findings reported by Tungbulu *et al.,* (2016) with respect to alkaloids and tannins, but were at variance with this latter report with respect to saponins and flavonoids. In contrast to findings of this present work, Etebu and Tungbulu (2016) had reported that postharvest period do not significantly (*P=0.05*) affect the amount of saponins and flavonoids of postharvest *Irvingia* fruits. In tandem with findings of this present work, Adebayo *et al* (2010) whilst working with pepper fruit (*Dennettia tripetala*) observed an increase in total phenol content of the fruit as storage time increased. Although the reason behind the increase could not be incontrovertibly deduced, these earlier findings validates the correspondent increase in alkaloid content of *Irvingia*

fruits as postharvest period increased. The concentration of phytochemicals in fruits such as apples has been reported to be dependent on a variety of factors, such as cultivar of the fruit, fruit part, harvest and storage conditions of fruits, as well as method processing of fruits (Boyer and Liu, 2004).

Table 1: The comparative effect of Postharvest period on weight, disease, microbial load, proximate and phytochemical contents of postharvest *Irvingia* fruit wastes

	Mean			Grand
Parameter	DAH = 0	DAH = 3	DAH = 6	**Mean**
Weight	51.55^{c}	43.56^{b}	34.57^{a}	42.23
Brownish black rot disease	4.35^{a}	23.56^{b}	76.48^{c}	34.8
Bacterial population	7.24×10^{6a}	1.29×10^{7b}	1.12×10^{7b}	1.04×10^{7}
Fungal population	4.57×10^{5a}	8.31×10^{5b}	1.05×10^{6c}	7.79×10^{5}
Moisture	85.75^{c}	82.81^{b}	81.54^{a}	83.37
Protein	0.65^{b}	0.69^{b}	0.58^{a}	0.64
Lipid	0.14^{c}	0.12^{b}	0.11^{a}	0.12
Fibre	0.83^{c}	0.78^{b}	0.62^{a}	0.74
Carbohydrate	12.08^{a}	14.88^{b}	15.94c	14.30
Alkaloid	3.23^{a}	5.17^{b}	6.18^{c}	4.86
Tannin	1.77^{a}	4.38^{b}	5.34^{b}	3.83
Saponin	1.45^{a}	2.17^{b}	2.08^{b}	1.90
Flavonoid	3.51^{a}	4.89^{b}	4.35^{b}	4.25

****** Means followed by different letters within rows are significantly different at the 5% probability level

Table 2: Correlation matrix weight, disease, microbial population, proximate and phytochemical contents of postharvest *Irvingia* fruit wastes

Parameters	Weight	Disease	Bacterial Population	Fungal population	Moisture	Protein	Lipid	Fibre	Carbohydrate	Alkaloid	Tannin	Saponin
Disease	-0.99**											
Bacterial Population	-0.53	0.49										
Fungal population	-0.93**	0.90**	0.76*									
Moisture	0.94**	-0.91**	0.75*	-0.96**								
Protein	0.63	-0.71*	-0.07	-0.46	0.43*							
Lipid	0.92**	-0.93**	-0.61	-0.90**	0.90**	0.58						
Fibre	0.96**	-0.99**	-0.41	-0.86**	0.84**	0.79*	0.91**					
Carbohydrate	-0.95**	0.91**	0.70*	0.96**	-0.99**	-0.38	-0.89**	-0.84**				
Alkaloid	-0.97**	0.94**	0.70*	0.99**	-0.98**	-0.49	-0.92**	-0.89*	0.99**			
Tannin	0.94**	0.90**	0.76*	0.98**	-0.99**	-0.42	-0.88**	-0.84**	0.99**	0.99**		
Saponin	-0.75*	0.67*	0.78*	0.84**	-0.91**	-0.04	-0.73*	-0.56	0.92**	0.87**	0.90**	
Flavonoid	-0.51	0.43	0.95**	0.72*	-0.76*	0.14	-0.56	-0.32	0.73*	0.69*	0.76*	0.88**

*=Significant at *P=0.05*
**=Significant at *P=0.01*
Figures without asterisks indicate non significance at 5% probability level

Correlation between Microbial population, brownish black rot disease and proximate contents of postharvest *Irvingia* fruits

Correlation analyses result between microbial population and brownish black rot disease was dependent on type of microorganisms. Whilst the relationship between bacterial population and postharvest disease of *Irvingia* fruits was not significant at *P=0.05*, fungal population was significantly (*P≤0.01*), directly related to disease (Table 2). The lack of significant relationship between bacterial population and brownish black rot disease of postharvest *Irvingia* fruits at *P=0.05* suggests that bacteria do not play a primary role in the emergence and development of the disease. It is, however, pertinent to note that several bacterial species, some of which are known pathogens of other crops, have been isolated from postharvest *Irvingia* fruits (Etebu and Tungbulu, 2015).

In contrast to bacterial population, fungal population was observed to be significantly (*P≤0.01*), positively correlated to disease (Pearson Correlation Coefficient = 0.90) (Table 2). Whilst species of several fungal genera such as *Aspergillus*, *Penicillium*, *Botrytis*, *Rhizopus* and *Mucor* have been shown to associate with decaying *Irvingia* fruits (Etebu, 2012, 2013; Joseph and Aworh, 1992), only *Botrytis* species has been reportedly proven to be the causative agent of brownish black rot disease in postharvest *Irvingia* fruits (Joseph and Aworh, 1992).

It is interestingly to note that fungal population revealed a significant (*P≤0.05*), direct relationship with bacterial population (Table 2). Although bacteria may not have played a primary role in the emergence and development of brownish black rot disease of postharvest *Irvingia* fruits, the significant increase in bacterial population after harvest (Table 1) coupled with its direct relationship with fungal population, indicates that the primary metabolic

activities of fungi on the fruits would alter the biochemical environment of the fruits in ways that would either sustain the growth of bacteria as observed in this work (Table 1) or inhibit their proliferation as reported by Etebu and Tungbulu (2015), depending on the suite of fungi involved.

Different components of proximate contents of postharvest *Irvingia* fruits were separately significantly ($P \leq 0.05$) correlated to bacterial and fungal populations enumerated on the fruits. Specifically, moisture content of the fruits was observed to be positively correlated to bacterial population (Pearson correlation coefficient = 0.75) whilst being significantly negatively correlated to fungal populations (Pearson correlation coefficient = -0.96) (Table 2).

Correlation analyses showed that the relationship of protein to either bacterial or fungal populations were not significant at $P=0.05$. Lipid was significantly ($P \leq 0.01$), negatively related to fungal population (Pearson correlation coefficient = -0.90) whilst its relationship with bacteria population was not significant at the 5% probability level (Table 2). This shows that an increase in fungal population present in postharvest *Irvingia* fruits led to a corresponding decrease in the amount of lipids contained in the fruits (Table 2), and vice versa. This result further suggests that the fungal population may have used the lipid content of the fruits for its metabolism and growth, and this led to steady depletion of lipids whilst favouring the proliferation of the fungal population.

Similar to lipid, fibre content of *Irvingia* fruits was significantly ($P \leq 0.01$), negatively related to fungal population (Pearson correlation coefficient = -0.86) whilst its relationship with bacteria population was not significant at the 5% probability level (Table 2). Dietary fibres are the sum total of plant polysaccharides indigestible by mammalian endogenous digestive enzymes (Theander et al., 1994), but fungi contain enzymes that are able to degrade virtually all kinds of polysaccharide (Kader and Barrett, 2005; Van den Brink and de Vries, 2011).

Carbohydrate was significantly ($P \leq 0.05$), positively related to bacterial and fungal populations (Table 2). The positive correlation of Carbohydrate to both types of microorganisms was expected being a nutritional requirement for majority of microorganisms (bacteria and fungi alike), needed for energy related metabolic processes.

Furthermore, correlation-regression analyses also revealed a significant ($P \leq 0.05$) relationship between brownish-black rot disease and different proximate components (Table 2). Findings showed that brownish black rot disease of *Irvingia* fruits was separately, significantly related to moisture, protein, lipids, fibre and carbohydrate (Table 2), indicating that postharvest disease of *Irvingia* is, to a large extent dependent on the amount and fate of the different proximate components of the fruits. Results showed that postharvest disease is inversely, significantly (($P \leq 0.05$)) related to protein content (Pearson correlation coefficient = -0.71) of *Irvingia* fruits (Table 2). This suggests that factors and conditions that favour disease progression among postharvest *Irvingia* fruits would also reduce the amount of protein contained in the fruits. Several reports show the apparent inverse relationships that exist between protein content and disease of fruits. In particular, Adeniyi *et al* (2014) showed that protein contents inherent in healthy *Irvingia* seeds (kernels) are significantly higher than their diseased counterparts. Also, Nweke and Ibiam (2012) had earlier on showed that fruits with soft rot disease have lower protein content in their pulp when compared to healthy fruits.

Similarly, brownish black rot disease was observed to be significantly (*P≤ 0.01*), inversely related to dietary fibre (Pearson correlation coefficient = -0.99). This finding is also in tandem with the results of the pioneering work of Etebu and Tungbulu (2016). The inverse relationship between brownish black rot disease and fibre content of *Irvingia* fruits is further corroborated by the work of Nweke and Ibiam (2012) who showed that *Annona muricata* (commonly called soursop) fruits plagued by soft rot disease had a relatively lower amount of fibre in their fruit pulp, compared to the their healthy counterparts. The existence of an inverse relationship between brownish black rot disease and fibre content of *Irvingia* fruits indicates that a decrease in fibre content would lead to a corresponding increase in disease and vice versa. However, a close look at the interrelationship between fungal population, disease and fibre suggests that a decrease in fibre may not have been necessarily responsible for the correspondingly increase in disease as observed in this work. Rather, it could be hypothesized that both fibre and brownish black rot disease responded separately to the activities of the fungal population. This position is predicated on the fact that fibre content decreased significantly as postharvest period increased whilst fungal population increased. Fungi are endowed with enzymes that enable them to degrade all forms of polysaccharides (Kader and Barrett, 2005; Van den Brink and de Vries, 2011) including fibre. With this obvious capability, fungi associated with the postharvest fruits could have utilized the fibre content of the fruits leading to the progressive depletion of the latter whilst fungal population blossomed. The increase in fungal population in turn could have resulted to an increased pathogenic potential which was evidenced in the corresponding increase of brownish black rot disease (Table 2).

Correlation between Microbial population, disease and phytochemicals of postharvest *Irvingia* fruits

Both populations of bacteria and fungi were separately observed to have a significantly (*P≤0.05*), direct relationship with all phytochemicals (Alkaloids, tannins, saponins and flavonoids) assessed in this work (Table 2). Brownish black rot disease was observed to have a significantly (*P≤0.01*), direct relationship with alkaloids, tannins and saponins but its relationship with flavonoids were not significant at *P=0.05* (Table 2). A close look at the correlation results further revealed that, apart from flavonoids, the relationship between individual phytochemical groups were more profound with the fungal population (*P≤0.01*) than their bacterial counterpart (*P≤0.05*) (Table 2).

Amongst several roles and functions, alkaloids and tannins are known to militate against the activities of pathogens in plants, and thus protect their host plants from microbial invasion (Ashihara *et al*, 2008; Wink, 1998). The separate positive relationships between brownish black rot disease with alkaloids and tannins may be the result of the over-all inter-relationship between the *Irvingia* fruit and disease causing fungal pathogens, and this probably explains why an increase in the population of microorganisms led to a significantly (*P≤0.05*), corresponding increase in alkaloids and tannins.

Similar to findings of Tungbulu *et al.,* (2016), alkaloids concentration was observed to be significantly (*P≤0.01*), positively related to tannins (Pearson correlation coefficient = 0.99) (Table 2). However, correlation/regression results between alkaloids and saponins as well as between alkaloids and flavonoids were clearly at variance from results reported by Tungbulu *et al*., (2016). Whilst Tungbulu *et al*., (2016) reported a no significant relationship between these groups of phytochemicals at *P=0.05*, results from this present work showed that alkaloids and saponins as well as between alkaloids and flavonoids showed significant (*P≤0.05*), direct relationships (Table 2). Also, whilst Tungbulu *et al*., (2016) showed that the

relationship between flavonoids and saponins were not significant at the 5% probability level, correlation analysis between these groups of phytochemicals as obtained from this present work revealed a significant (*P≤0.01*), direct relationship (Pearson correlation coefficient = 0.88) (Table 2). Discrepancies in correlation/regression analyses results between different phytochemicals amongst different workers are not new. For example whilst alkaloids had a significantly (*P≤ 0.05*) direct relationship with tannin in this present work, similar to earlier findings of Tungbulu *et al.*, (2016), Janzen and Waterman (1984) reported a negative correlation between these phytochemicals contents in plants. Numerous factors such as plant genotype, maturity, environment and postharvest conditions have been implicated with the bioavailability of phytochemicals (Manach *et al*., 2004; Boyer and Liu, 2004; Schreiner and Huyskens-Keil, 2006.

Inter-relationship between proximate contents and phytochemicals of postharvest *Irvingia* fruits

The proximate composition of food which has been reported by various workers to include its protein, carbohydrates, lipids, moisture and dietary fibre contents (Onimawo and Egbekun, 1998; Ihekoronye and Ngoddy, 1985) were studied in this work. Phytochemicals assessed on the other hand were alkaloids, tannins, saponins and flavonoids. Results in this work showed that several proximate parameters were significantly (*P≤ 0.05*) related to some or all phytochemicals (Table 2).

In particular, results showed that moisture was significantly, negatively correlated to all phytochemicals assessed in this work (Table 2) indicating that a decrease in moisture led to a corresponding increase in amounts of all the different phytochemicals. Whilst moisture showed a negative relationship with all the phytochemicals, carbohydrate showed significant positive relationships with all the different phytochemicals assessed in this work. This means that an increase in carbohydrate in postharvest *Irvingia* fruits led to a corresponding increase in alkaloids, tannins, saponins and flavonoids. The separate positive relationship between carbohydrate and all phytochemicals assessed in this work suggests the existence of some sort of affinity or bonding between them. Findings from a few workers further give credence to this position. In particular, most phytochemicals have been reported to occur in plants as conjugates with sugars (Shahidi and Wanasundara, 1997; Wyen *et al*., 2000).

Fibre content showed a significant (*P≤ 0.05*) negative relationship with alkaloids and tannins but not with saponins and flavonoids. Correlation between fibre and either of these latter phytochemicals were not significant at 5% probability level (Table 2). Protein showed no significant correlation to any of the phytochemicals at the 5% probability level. This shows that protein may not be related to any of the phytochemicals assessed in this work.

Lipids, similar to moisture, were observed to have significant negative relationship with all phytochemicals except flavonoids (Table 2). Studies centered on correlation between proximate contents and phytochemicals of fruits are fairly scanty. However, a recent study of a fungus by Olteanu *et al*., (2010) showed that biosynthesis of crude lipid either remained stable or diminishes after alkaloid biosynthesis begins. This report gives credence to the negative relationship observed to exist between lipids and alkaloids in this work. Whilst carbohydrate was observed to correlate significantly (*P≤0.05*), positively with all phytochemicals, lipids were observed to be significantly (*P≤0.05*), negatively related to all phytochemicals except flavonoids. Reports had also shown that most phytochemicals also occur as conjugates of lipids, amongst other proximate contents in plants (Shahidi and Wanasundara, 1997; Wyen *et al*., 2000).

Whilst proximate contents measure the nutritional quality of foods, phytochemicals in plants have been shown to confer numerous health benefits to consumers. Although a few works had studied the effect of postharvest storage period on these parameters in postharvest *Irvingia* fruits (Etebu and Tungbulu, 2016, Etebu *et al.*, 2016; Tungbulu *et al.,* 2016), the potential relationship that exists between proximate contents and phytochemicals of postharvest *Irvingia* fruits have not been studied; these were studied for the first time in this present work. The significant correlations observed in this work between some proximate contents (particularly Carbohydrate and Lipids) and phytochemicals of postharvest *Irvingia* fruits is a clear indication that phytochemicals most probably occur as conjugates of proximate contents. This position is well documented among plants (Shahidi and Wanasundara, 1997; Wyen *et al*., 2000). Furthermore, phytochemicals have been shown to interact non-covalently with macronutrients such as carbohydrate, proteins lipids etc, and these interactions potentially affect the availability and reactivity of phytochemicals in foods and guts, and hence their functions (Bordenave *et al*., 2014). Also, the sensory characteristics (taste, flavor, texture, and color) of foods of plant origin are reportedly influenced by interactions between phytochemicals and macronutrients such as lipids, carbohydrates, proteins etc. These apparent interactions between proximate contents and phytochemicals would probably explain the difference in taste between the two prominent species of *Irvingia* (*Irvingia gabonensis* and *I. wombolu*) in Nigeria.

Conclusion

The health and growth benefits of fruit consumption accruable to humans and animals apparently depends on the type and amount of proximate and phytochemical content inherent in the fruits

Although the bioavailability of phytochemicals in plants has been reported to be influenced by a variety of factors such as genotype, maturity, environment and postharvest conditions (Manach *et al*., 2004; Boyer and Liu, 2004; Schreiner and Huyskens-Keil, 2006), results obtained from this work showed that all the different groups of phytochemicals were significantly ($P \leq 0.05$) interrelated to one another (Table 2), as well as to some or all proximate contents. It does appear that the overall proportion of proximate contents and phytochemical is driven by the overall growth and metabolic needs of the fruits vis-à-vis the potential threat of pathogens and prevailing unfavourable environmental conditions. It would be interesting therefore to understand how biotic and abiotic factors influence the biosynthetic processes in plants to maintain the right mix/balance of proximate contents and phytochemicals in *Irvingia* fruits.

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References

Adebayo BC, Oboh G, Akindahunsi AA (2010). Changes in the total phenol content and antioxidant properties of pepperfruit (*Dennettia tripetala*) with ripening. *Afr. J. Food Sci.* 4 (6): 403-409.

Adeniyi SAA, Adedotun, AA and Akinniyi O (2014). The effects of post harvest mycodeterioration on the proximate composition of irvingia gabonensis seeds. *Int. J. Phytopathol.* **03(01):** 41-48

Akubor PI (1996). The suitability of African bush mango juice for wine production. *Plant*

Foods and Human Nutrition. **49:** 213–219.
Association of Official Analytical Chemists, (1986). Official methods of analysis, 16th ed., Washington DC, U.S.A.
Association of Official Analytical Chemists, (2000). Official methods of analysis, 17th ed., Gaithersburg, MD, USA, Official Method. 999. 11.
Ashihara H, Sano H, Crozier A (2008). Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. *Phytochem*. 69:841–856.
Ayuk ET, Duguma B, Franzel S, Kengue J, Mollet SM, Tiki-Manga T and Zenkekeng P (1999). Uses, management and economic potentials *Irvingia gabonensis* in the humid lowlands of Cameroon. *For. Ecol. Manage*. **113**: 1-9
Boakye AA, Wireko-Manu FD, Agbenorhevi JK and Oduro I (2014). Dietary fibre, ascorbic acid and proximate composition of tropical underutilized fruits. *Afr. J. Food Sci.* **8** (6): 305-310
Bordenave N, Hamaker BR, Ferruzzi MG (2014) Nature and consequences of non-covalent interactions between flavonoids and macronutrients in foods. *Food Funct* 5:18–34
Boyer J and Liu RH (2004). Apple phytochemicals and their health benefits. *Nutr. J*. 3: 5
Cutsem V (1989). The in-vitro antifungal spectrum of Itraconazole. *Mycoses.* **32**, Suppl. 1: 7-13
Etebu E (2012). Postharvest pathology and phytochemicals of *Irvingia gabonensis (Aubry- Lecomte ex O'Rorke)* fruits and wastes. *Agric.Sci.Res.J*, **2**(6): 285-294
Etebu E (2013). Differences in Fruit size, Post harvest pathology and phytochemicals between *Irvingia gabonensis and Irvingia wombolu. Sust. Agric. Res.* **2** (1): 52-61
Etebu E and Tungbulu G. (2015). Bacterial quality of postharvest Irvingia gabonensis (*Aubry-Lecomte ex O'Rorke*) fruit wastes. *Int. J. Appl. Microbiol. Biotechnol. Res.* **3:** 96-103
Etebu, E and Tungbulu, G (2016) Effect of postharvest period on disease progression and proximate composition of Irvingia species fruit waste. *IOSR J. Environ. Sci., Toxicol. Food Techn*. 10: 26-36
Etebu E, Akani, N. P, Young-Harry, W and Ogagbe, G. (2009) The effect of drying temperatures on postharvest fungal colonization and populaton, and antibacterial activity of selected chewing sticks. *Nigerian Journal of Plant Protection*, **23:** 33-43
Etebu E, Tasie AA and Daniel-Kalio LA (2003). Post-harvest fungal quality of selected chewing sticks. *Oral dis.* **9** (2): 95-98
Etebu E, Tungbulu G and Ezenwaka J (2016). Effect of postharvest period on disease progression, weight and vitamin content of Irvingia species fruit wastes. *Int. J. Agric. Inn. Res.* 5 (1): 98-104
Eyeson KK and Ankrah EK (1975). Composition of foods commonly used in Ghana (pp. 15-17). Accra: Food Research Institute, CSIR. Ghana. UNDP/FAO Publication
Fagbohun ED and Faleye OS (2012). Effect of storage on chemical composition and mycoflora of okra (*Abelmoschus esculentus*). *Int. J. Biosci.* **2(7):** 83-89
Gomez KA and Gomez AA (1984). *Statistical procedures for agricultural research* 2nd edition. John Wiley and Sons, N.Y. 680pp.
Gopalan C, Ramasastri BV, Balasubramanian SC (2000). Proximate principles: Common foods. In: Nutritive value of Indian foods (Revised and Updated Edition). Narasinga Rao BS, Pant KC, Deosthale YG, (Eds.), National Institute of Nutrition, ICMR, Hyderabad, India. pp: 53-55.
Guéguen, J. 1991. Pea and fababean proteins. In: Hudson, B. J. F. (ed.) *Development of food proteins. Elsevier Applied Sciences*, London and New York. Vol. 7: 35-78
Harbone JB (1973). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall, Ltd. London 278pp

Harris DJ (1996). A revision of the *Irvingiacea* in Africa. *Bulletin du Jardin Botanique National de Belgique* **65:** 143-196.

Ihekoronye AI, Ngoddy PO (1985). *Integrated food science and technology for the Tropics*. Macmillan Publishers Ltd. London. pp. 283-294.

Janzen DH and Waterman PG (1984). A seasonal census of phenolics, fibre and alkaloids in foliage of forest trees in Costa Rica: some factors influencing their distribution and relation to host selection by Sphingidae and Saturniidae. *Biol. J. Linn. Soc*. **21:** 439-454.

Joseph K and Aworh OC (1991). Composition, sensory quality and respiration during ripening and storage of edible wild mango (*Irvingia gabonensis). Int. J. Food Sci. Technol.* **26**: 337-342.

Joseph K and Aworh OC (1992). Post-harvest treatment of wild mango (*Irvingia gabonensis*) for improved shelf life. *Food Chem*. **44:** 45-48.

Kader AA and Barrett DM (2005). Classification, composition of fruits and postharvest maintenance of quality. In: Barrett, DM., Somogyi L and Ramaswamy H (eds.) Processing Fruits: Second Edition: Science and Technology . CRC Press, Boca Raton, FL. P. 3-22

Ladipo DO, Fondoun JM and Gana N (1996). Domestication of the bush mango (*Irvingia spp*): Some exploitable intra-specific variation in West and Central Africa. In: Domestication and Commercialization of Non-Timber Forest Products for Agroforestry (eds.) Leakey, R.R.B., Temu, A.B., Melnyk, M. and Vantomme, P. Non-Timber Forest Products Paper 9, FAO Rome. Pp 193-206.

Leakey R, Schrecheriberg K and Tchoundjev Z (2003). The participatory domestication of West African indigenous fruits. *Int. For. Rev.*, **5:** 338-347

Lowe AJA, Gillies CM, Wilson J and Dawson IK (2000). Conservation genetics of bush mango from central/west Africa: Implications from random amplified polymorphic DNA analysis. *Mol. Ecol*. **9:** 831-841.

Mamiro P, Fweja L, Chove B, Kinabo J, George V and Mtebe K (2007). Physical and chemical characteristics of off vine ripened mango (*Mangifera indica* L.) fruit (Dodo), *Afri. J. Biotech.* **6(21):** 2477-2483.

Manach C, Scalbert A, Morand C, Rémésy C and Jiménez L (2004). Polyphenols: Food sources and bioavalability. *Amer. J. Clinical Nutr.* **79:** 727-747

Matos L, Nzikou JM, Matouba E, Pandzou-Yembe VN, Mapepoulou TG, Linder M and Desobry S (2009). Studies of *Irvingia gabonensis* seeds kernels: Oil technological applications. *Pak. J, Nutr*. **8:** 151-157

Ngondi JL, Oben JE and Minka SR (2005). The effect of *Irvingia gabonensis* seeds on body weight and blood lipids of obese subjects in Cameroon. *Lipids in health and Disease.* **4:** 12-15

Nixwell MF, Johanna MP and Ngezimana W (2013). Proximate, chemical compositions and sulphur concentrations on quality of selected dried mango (*Mangifera indica* L.). Afr. J. Biotechnol. **12(19):** 2678-2684. DOI: 10.5897/AJB2013.12256.

Nweke CN and Ibiam OFA (2012). Pre and post harvest fungi associated with the soft rot of the fruit of *Annona muricata*, and their effects on the nutrient content of the pulp. *Am. J. food Nutri.* **2(4):** 78-85

Okafor JC (1985). Selection and improvement of indigenous tropical fruit tree: problems and prospects. *J. Forest Res*., **1:** 87-95

Olteanu Z, Surdu Ş, Roşu C, Truţă E, Zamfirache MM and Oprică L (2010). Dynamics of alkaloid biosynthesis in correlation With lipid biosynthesis in submerged cultivated Strains of *Claviceps purpurea*. Analele Stiintifice ale Universitatii "Alexandru Ioan

Cuza" din Iasi Sec. II a. Genetica si Biologie Moleculara, **11:** 2248-3276. Available at: <http://www.gbm.bio.uaic.ro/index.php/gbm/article/view/835>. Accessed: 03 January, 2017.

Onimawo IA, Egbekun MK (1998). *Comprehensive food science and nutrition*. Revised edition. Ambik Publishers, Benin City.

Onimawo IA, Oteno F, Orokpo G and Akubor PI (2003). Physicochemical and nutrient evaluation of African bush mango (*Irvingia gabonensis*) seeds and pulp. *Plant foods for Hum. Nutri.* **58:** 1-6

Osborne DR and Voogt P (1987): The Analysis of Nutrients in food. Academic Press, London. pp 130-238.

Prusky D and Keen NT (1993). Involvement of preformed antifungal compounds in the resistance of subtropical fruits to fungal decay. *Plant Dis*. **77:** 114 - 119

Schreiner M and Huyskens-Keil S (2006). Phytochemicals in fruit and vegetables: Health promotion and postharvest elicitors. *Crit. Rev. in Plant Sci.* **25:** 267–278

Shahidi F and Wanasundara U (1997). Measurement of lipid oxidation and evaluation of antioxidant activity. In: Shahidi F (Ed.) *Natural Antioxidants, Chemistry, Health Effects and Applications*, AOCS Press Champaign IL, USA.

Shiembo PN, Newton AC and Leakey RRB (1996). Vegetative propagation of *Irvingia gabonensis*, a West African fruit tree. *For. Ecol. Manage.* **87:** 185-192

Sofowora A (1993). *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria, 289pp

Swain T (1979). Tannins and Lignins. In: Rosenthal, GA and Janzen DH (Eds.) *Herbivores: their interactions with plant metabolites.* Academic Press, New York.

Theander O, Åman P, Westerlund E and Graham H (1994). Enzymatic/chemical analysis of DF. J. AOAC Int. **77:** 703-709.

Trease GE and Evans WC (1989). *Pharmocognosy*.11th edition. Brailliar Tiridel Can, Macmillan publishers

Tungbulu G, Etebu E and Ezenwaka J (2016). Effect of postharvest period on phytochemical content and brownish-black rot disease of postharvest Irvingia species fruit wastes. *Int. J. Agric. Inn. Res.* 5 (1): 105-112

Turner, S. R. Barrat, D. H. P. and Casey R. 1990. The effect of different alleles at the *r* locus on the synthesis of seed storage proteins in *Pisum sativum. Plant Mol. Biol.* **14:** 793 803

Van den Brink, J., and de Vries, R. P. 2011. Fungal enzyme sets for plant polysaccharide degradation. *Applied Microbiology and Biotechnology*, **91(6):** 1477–1492. http://doi.org/10.1007/s00253-011-3473-2

Wink M (1988). Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theor. Appl. Genet*. **75:** 225-233

Wyen D, Takacsova M, Jakubik T and Dang M (2000). Antioxidant effects of thyme in rape seed oil. *Biologia Bratislava*. **55:** 277-281.

Zhishen J, Mengcheng T and Jianming W (1999). The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* **64:** 555 -559