

## Fungal quality and phytochemicals of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) kernels sold in Yenagoa metropolis of Bayelsa State, Nigeria

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### Abstract

The kernels of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) are highly valued for their nutritional and health benefits but they are susceptible to storage fungi that could evoke a broad range of toxic properties. Although *Irvingia* kernels are widely consumed in Bayelsa State the potential hazards and benefits of *Irvingia* consumption in the State has not been studied. Hence the fungal quality (potential hazards) and phytochemicals (potential benefits) of *Irvingia* kernels sold in selected markets (Swali, Opolo and Edepie) in the State was investigated. Results showed that *Irvingia* kernels were sold in open plastic containers. Fungal population on the kernels on display for sale varied significantly ( $P \leq 0.05$ ) among the selected markets. Mean Fungal population per gram of *Irvingia* kernels obtained from Swali, Opolo and Edepie markets were  $1.17E+05$ ,  $2.40E+05$  and  $6.92E+05$  respectively. Five genera (*Rhizopus*, *Mucor*, *Aspergillus*, *Penicillium* and *Candida*) of fungi were isolated across all three markets. Phytochemical analyses showed that *Irvingia* kernels sold in all markets under study possessed alkaloids, saponins, tannins and flavonoids, known to play significant roles in human medicine. Some of the fungi found to colonize the kernels have been implicated in the production of potentially potent toxins under suitable conditions. We recommend that *Irvingia* kernels on sale should be monitored regularly by relevant government agencies to ensure the safety of consumers, whilst the conditions under which postharvest fungi would produce mycotoxins in *Irvingia* kernels during storage are studied.

**Keywords:** *Irvingia gabonensis*, Postharvest fungi, *Aspergillus* spp., *Penicillium* spp., Phytochemicals, Fungal quality

### 1. Introduction

*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke), sometimes called Bush Mango is a dominant tropical forest tree of west and Central Africa, which is rated as one of the most domestically consumed wild fruit tree (Ladipo *et al.*, 1995). In Nigeria, particularly in the southern and Eastern regions, it is grown and consumed basically because of its kernels which serve as a major condiment in the preparation of Nigeria's famous 'Ogbono' soup. The department of Forest Resource Management in Ibadan (1986) estimated that annual consumption of *Irvingia* seeds in southern regions of Nigeria ranged from 3.2 to 14.13 kg per household. Similarly, Oyakhilome (1985) estimated that *Irvingia* seeds are used in one third of all soup preparations in Nigeria's southern region.

*I. gabonensis* is highly valued for its health and medicinal benefits (Duguma *et al.*, 1990; Ndoye *et al.*, 1997), and agricultural potentials (Etebu, 2012a). Studies have shown that seed extract of *I. gabonensis* caused a significant reduction in body weight among obese people in Cameroon (Ngondi *et al.*, 2005). Similarly, earlier studies had attributed a reduction in the incidence of diseases such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction to consumption of fruits and vegetables (Ames *et al.*, 1993; Liu, 2003; Vinson *et al.*, 1998), which in turn was credited to natural antioxidant phytochemicals inherent in them (Quideau *et al.*, 2011). Agbor and associates (2005) while working with various herbs and spices showed that *Irvingia* seeds had high antioxidant capacity.

The downside to all these potential benefits derivable from consuming *Irvingia* kernels is that they are highly susceptible to postharvest spoilage fungi while in storage. In particular, several studies have shown that *Irvingia* kernels displayed on shelves for sales in Nigerian markets are often contaminated with spoilage fungi (Adebayo-Tayo *et al.*, 2006; Iyayi *et al.*, 2010), some of which produce mycotoxins. Mycotoxins are toxic secondary

metabolites common in many grains during storage and are produced under appropriate environmental conditions by filamentous fungi, mainly *Aspergillus spp.*, *Penicillium spp.*, and *Fusarium spp.* (Amadi and Adeniyi, 2009; Jestoi, 2008).

Consumption of high levels of mycotoxins can evoke a broad range of toxic properties including carcinogenicity, neurotoxicity, as well as reproductive and developmental toxicity (Jestoi, 2008). In particular, aflatoxin, a common mycotoxin associated with food, has been reported to have caused illness among several hundreds of Kenyans in 2004, and leaving 125 people dead (Lewis *et al.* 2005; Strosnider *et al.* 2006). It has been estimated that more than 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Strosnider *et al.* 2006).

Although *Irvingia* seeds are of great importance in Nigeria, especially in the South-south region where the tree is found to be abundant, the potential fungal hazards among consumers of *Irvingia* kernels in Bayelsa State has not been studied. Furthermore, although Agbor and associates (2005) had shown that *Irvingia* seeds possess high antioxidant capacity, no work (to the best of our knowledge) has been done to assess the presence of specific groups of phytochemicals thought to confer antioxidant capacity on *Irvingia* kernels. Hence in this research, the fungal quality and phytochemicals of *Irvingia gabonensis* (Aubry-Lecomte Ex O'Rorke) kernels sold in Yenagoa, Bayelsa State capital of Nigeria was studied. Findings of this study would afford us the prerequisite knowledge to assess the potential benefits and dangers of consuming *Irvingia* kernels.

## 2. Materials and methods

### 2.1 Assessing fungal population and quality of *Irvingia* kernels

About 100g of *Irvingia* kernels were separately bought from three retailers in Swali market (Fig. 1), Yenagoa metropolis located at Latitude 4°55'29"N and Longitude 6°15'51"E of Bayelsa State, and thoroughly mixed to form a composite sample. Thereafter, the kernels were ground with a domestic electric blender and 10g was suspended in saline water to arrive at a final volume of 100ml. Thereafter, 1ml of *Irvingia* kernels suspension was serially diluted and plated in 3 replicates onto potato dextrose agar (Oxoid Ltd, Hampshire, UK) previously prepared according to manufacturer's prescription, and integrated with 50µg ml<sup>-1</sup> each of streptomycin and tetracycline according to Etebu *et al.* (2003). The plates were thereafter incubated at ambient room temperature for 3 days. At the end of 3 days colony forming units were counted and the fungal population was expressed as colony forming units per gram of *Irvingia* kernel. Colonies were thereafter repeatedly subcultured after every three days onto newly prepared agar plates until pure cultures were obtained. Fungal colonies were thereafter transferred onto Sabouraud dextrose agar (Oxoid Ltd, Hampshire, UK) and incubated in a sporulating chamber under black light for 3 days (adapted from Etebu *et al.*, 2005), and identified based on macroscopic and microscopic examination according to Alexopoulos (1962) and Barnett and Hunter (1972). The experiment was separately repeated with *Irvingia* kernels bought from Opolo and Edepie markets respectively. Fungal population data was log transformed according to Gomez and Gomez (1985), and subjected to ANOVA using Generalized Linear Model of SPSS version 16.0, Statistical software. Mean log colony forming units were further subjected to Tukey's mean separation test, and de-transformed (weighted) prior to discussion.

### 2.2 Qualitative analysis of phytochemicals

The ground *Irvingia* kernels from the different markets were separately screened in different ways (depending on phytochemical) to determine the presence of various phytochemicals (Alkaloids, flavonoids, saponins, tannins and glucosides) according to Harborne (1973), Trease and Evans (1989) and Ukoha *et al.*, (2011).

**Test for alkaloids:** Two grams of ground *Irvingia* kernels was boiled in 5ml of 2% Hydrochloric acid (HCl) on a steam bath for 5mins. The mixture was allowed to cool and filtered. To 2ml of filtrate was added 1ml of Mayer's reagent. The formation of a creamy white precipitate indicated the presence of alkaloids.

**Test for flavonoids:** 10ml of ethyl acetate was added to 5g of ground *Irvingia* kernels in a test tube, and brought to boiling for 1 minute in a water bath. The mixture was filtered and to 4ml of the filtrate was shaken with 1ml of 1%

Aluminium Chloride ( $AlCl_3$ ). This was allowed to stand for 15 minutes for colour development. The formation of a yellow colour in the presence of 1ml of ammonia solution indicated the presence of flavonoids.

Test for saponins: 5ml of distilled water was added to 1g of ground *Irvingia* kernels in a test tube, and boiled in a water bath for 5 minutes. The mixture was filtered when still hot, and to 1ml of the filtrate, 2 drops of olive oil was added. The tube was thereafter corked and shaken vigorously for about 60 secs. The formation of emulsion indicated the presence of saponins.

Test for tannins: 5ml of 45% ethanol was added to 2g of ground *Irvingia* kernels in a test tube, and boiled for 5 minutes. The mixture was cooled and filtered. To 1ml of the filtrate, 5 drops of lead sub-acetate solution was added. The formation of a gelatinous precipitate indicates the presence of tannins. 1ml of filtrate was collected and 0.5ml of Bromine water was added, and the formation of a pale brown precipitate indicated the presence of tannins.

Test for glycosides: 30ml of distilled water was added to 2g of ground *Irvingia* kernels in a test tube, and heated for 5 minutes in a water bath. The resultant mixture was thereafter filtered. To 5ml of the filtrate, 0.2ml of Fehling's solution A and B was added and heated for 2 minutes in a water bath. The mixture was observation for a brick-brown/red colouration which would indicate the presence of glycosides.

### 3. Results and discussion

*Irvingia* kernels were observed to be stored in open plastic containers as they were being displayed for sale in the selected markets (Fig. 2). The way and manner the kernels were displayed for sale exposes them to environmental influences that would facilitate fungal contaminations. Contamination of food stuff has been reported to arise from insect's infestation, wound and presence of foreign matter such as sand, dust and debris among others (Djerbi, 1983). Prior to transportation to the market for sales, locals who engage in the business of *Irvingia* sales generally split the fruit to extract the kernels (Ladipo *et al.*, 1996; Ayuk *et al.* 1999; Etebu, 2012a), and dries the same under the sun until it is sufficiently dry (Ladipo, 1999). Damages on seeds caused by either insects or by way of handling have been shown to provide entry points for fungal infection (Dennis 2002). A close look at the kernels on display (Fig. 2) also revealed that some of the *Irvingia* kernels were damaged. It is therefore very likely that notwithstanding the drying of the kernels as a means of processing them to forestall postharvest spoilage, wounds inflicted on the kernels during extraction could have sufficiently aided fungal infection.

Results obtained from this work further showed that fungal population that colonized the kernels on display for sale varied significantly ( $P \leq 0.05$ ) among the selected markets within the Yenagoa metropolis of Bayelsa State, Nigeria. Mean Fungal population per gram of *Irvingia* kernels obtained from Swali, Opolo and Edepie were  $1.17E+05$ ,  $2.40E+05$  and  $6.92E+05$  respectively (Table 1). No clear deductions could be made to possibly explain the significant ( $P \leq 0.05$ ) differences in fungal population observed from kernels sold in the different markets. However, it would be worthwhile to note that Swali market is the major market in Bayelsa State and Yenagoa in particular. As a result, the sales of commodities are likely to have higher turn-over in this market than the rest. Additionally, locals who harvest the kernels would sell them in wholesale in Swali market and retailers would in turn buy from dealers in this market and sell them in other smaller markets within or outside the state. The kernels sold in Opolo and Edepie markets would therefore have been stored for longer periods after harvest. Several studies carried out quite recently have shown that fungal population on *Irvingia* fruits increased significantly as days of storage after harvest increased (Etebu, 2012b).

Five genera of fungi (*Rhizopus*, *Mucor*, *Aspergillus*, *Penicillium* and *Candida*) were isolated across all three selected markets under study (Table 1). *Rhizopus* and *Mucor* species were observed to be common to all markets, and they were the only group of fungi that were isolated from Swali market. Whilst, the kernels sold in Opolo were, in addition to *Rhizopus* and *Mucor* species also colonized by *Penicillium* and *Candida* species, those sold in Edepie were colonized by *Rhizopus*, *Mucor* and *Aspergillus* species (Table 1).

Species of all five genera of fungi isolated in this work have been associated with various plant and secondary food rot. Although they are usually not primary plant pathogens, they are often encountered as storage fungi on plant products (Kozakiewicz 1989; Perrone *et al.*, 2007; Ilondu, 2011; Kobina and Ebenezer, 2012; Etebu, 2012a). They infect plants and plant produce at different stages including pre-harvest, harvest, processing and handling.

The isolation of *Aspergillus* and *Penicillium* species isolated from *Irvingia* kernels sold in Opolo and Edepie markets calls for concern because members of these two fungal genera amongst others have been shown to produce mycotoxins (Adams, 1977). Although, no illness has been attributed to the consumption of *Irvingia* among those who consume the fruit/kernels in Bayelsa State or anywhere else, to the best of my knowledge, it is imperative to study the conditions under which toxigenic fungi could produce mycotoxins while colonizing *Irvingia* kernels in storage. This call is particularly necessary because aflatoxins have been detected in *Irvingia* kernels displayed for sale in some parts of Nigeria (Adebayo-Tayo *et al.*, 2006). Additionally, aflatoxins have been found to be heat stable (Frazier and Westhoff, 1988).

Consumers of fungal contaminated *Irvingia* kernels are therefore at risk of mycotoxigenic poisoning even if such kernels are cooked. It would be imperative for governmental Agencies, such as National Agency for food and Drug Control (NAFDAC) in Nigeria, saddled with the responsibility of ensuring food safety to regularly monitor and assess the potential risk factors of *Irvingia* kernels sold in our markets. It would suffice at this juncture to reiterate that the association of toxigenic fungi with agricultural products in storage such as grains may not necessarily result to formation of mycotoxins. Predisposing factors that influence the production of mycotoxins by toxigenic fungi in contaminated food are said to include postharvest conditions such as storage, transportation, food processing and environmental conditions (Jestoi, 2008; Wu and Khlanguiset 2010). It would be advisable to study the conditions that influence the production of mycotoxins by fungi contaminating *Irvingia* kernels in storage. A very recent, yet to publish, work by Etebu and Bawo (In press) showed that *Irvingia* kernels that were treated with NaCl or sun-dried and then stored in sealed polythene bags produced a relatively reduced amount of *Aspergillus flavus*, *Penicillium* and *Fusarium* species, known to produce mycotoxins than those treated differently of treated same way but stored in unsealed polythene bags. Although, mycotoxins were not assessed in this work, it does appear that the way and manner *Irvingia* kernels are displayed (Fig. 2) may encourage growth of toxigenic fungi.

Phytochemical analyses showed that *Irvingia* kernels sold in all markets under study possess alkaloids, flavonoids, saponins and tannins (Table 2). These groups of phytochemicals have been shown to play very significant roles in human medicine and treatment of ailments (Addae-Mensah, 1992; Bansa and Adeyemo, 2007; Duguma *et al.*, 1990; Raskin *et al.*, 2002; Rates, 2001). Fruits and vegetables are known to possess natural phytochemicals (Quideau *et al.*, 2011), and people who regularly consume them are less likely to suffer from diseases such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction (Ames *et al.*, 1993; Liu, 2003; Vinson *et al.*, 1998). Agbor and associates (2005) while working with various herbs and spices showed that *Irvingia* seeds had high antioxidant capacity. Findings from this present work have shown the presence of different phytochemicals in postharvest *Irvingia* kernels which would have contributed to the high antioxidant capacity observed by Agbor and associates (2005), and would have been responsible for the reduction in body weight among obese people in in Cameroon (Ngondi *et al.*, 2005). Further studies are required to substantiate these assumptions.

Alkaloids and saponins have been shown to possess antimicrobial capabilities (Ashihara *et al.*, 2008; Mert-Türk, 2006). Etebu (2012b), working on fungal quality of *Irvingia* fruits mesocarp reiterated that the relatively higher amount of phytochemicals in *I. wombolu* (bitter variety) potentially reduced the fungal population colonizing its fruits compared to *I. gabonensis* (sweet variety). Although saponins are known to play very important roles in human and animal nutrition, some of them have been reported to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia (Francis *et al.*, 2002). Saponins therefore could affect animals in a host of different ways both positive and negative. There is the need to further study the fate and persistence of phytochemicals in *Irvingia* kernels whilst in storage.

Tannins are one of the major phytochemicals found in many higher plants. Like saponins, they affect animals in ways that could be described as both positive and negative. For example, they are thought to greatly impact on animal nutrition, including inhibition of growth rate digestive enzymes (Bennick, 2002) because they are able to precipitate proteins through the effective formation of strong complexes with proteins. On the positive side, tannins have been associated with various pharmaco-therapeutic effects. In particular, tannins-rich remedies are used as antioxidants (Koleckar *et al.*, 2008), anthelmintics (Ketzis *et al.*, 2006), antimicrobials and antivirals (Buzzini *et al.*, 2008), in cancer chemotherapy (Chung *et al.*, 1998) and to chelate dietary iron (Clauss *et al.*, 2007).

Flavonoids also occur in most plants, and they are the pharmacologically active constituents in many herbal plant medicines (Parsaeimehr et al., 2011). Flavonoids have been shown to protect the gastrointestinal (GI) tract, having antispasmodic, antidiarrhoeal, antibacterial, antisecretory and antiulcer properties, as well as strong antioxidant capacities (Di Carlo et al., 1993; La Casa et al., 2000; Sunairi et al., 1994; Isomoto et al., 2005; Rice-Evans et al., 1997). Etebu (2012a) working on the phytochemicals inherent in *Irvingia* fruits mesocarp reiterated that these potential health enhancing properties of flavonoids would be contributing immensely to the health of locals in the villages across the Niger Delta region of Nigeria who regularly eat fresh *Irvingia* fruits as snack. Although locals who harvest the fruits sometimes eat the fresh fruits (Okafor, 1975; Leakey, 1999; Fajimi *et al.*, 2007), several workers noted that the common practice among the majority of locals is to split the fruit for its kernel (nut) while the fleshy mesocarp is thrown away and left to rot (Ladipo *et al.*, 1996; Ayuk *et al.* 1999; Etebu, 2012a). In contrast, the department of Forest Resource Management in Ibadan (1986) estimated that annual consumption of *Irvingia* kernels in southern regions of Nigeria ranged from 3.2 to 14.13 kg per household. Similarly, Oyakhilome (1985) estimated that *Irvingia* seeds are used in one third of all soup preparations in Nigeria's southern region. Hence the perceived health benefits arising from *Irvingia* consumption among locals within the southern region of Nigeria are more likely to be derived from the kernels than the fruit pulp.

#### 4. Conclusion

Findings from this study revealed that *Irvingia* kernels displayed for sale in Yenagoa metropolis of Bayelsa State, Nigeria possess secondary metabolites with potential health and nutritional benefits. However, they were also found to be susceptible to postharvest spoilage fungi while in storage. Some of the fungi found to colonize the kernels have been implicated in the production of potentially potent toxins under suitable conditions. It would be imperative for government agencies to regularly monitor and assess the potential risk of consuming *Irvingia* kernels whilst the conditions under which postharvest fungi would produce mycotoxins in *Irvingia* kernels during storage are studied.

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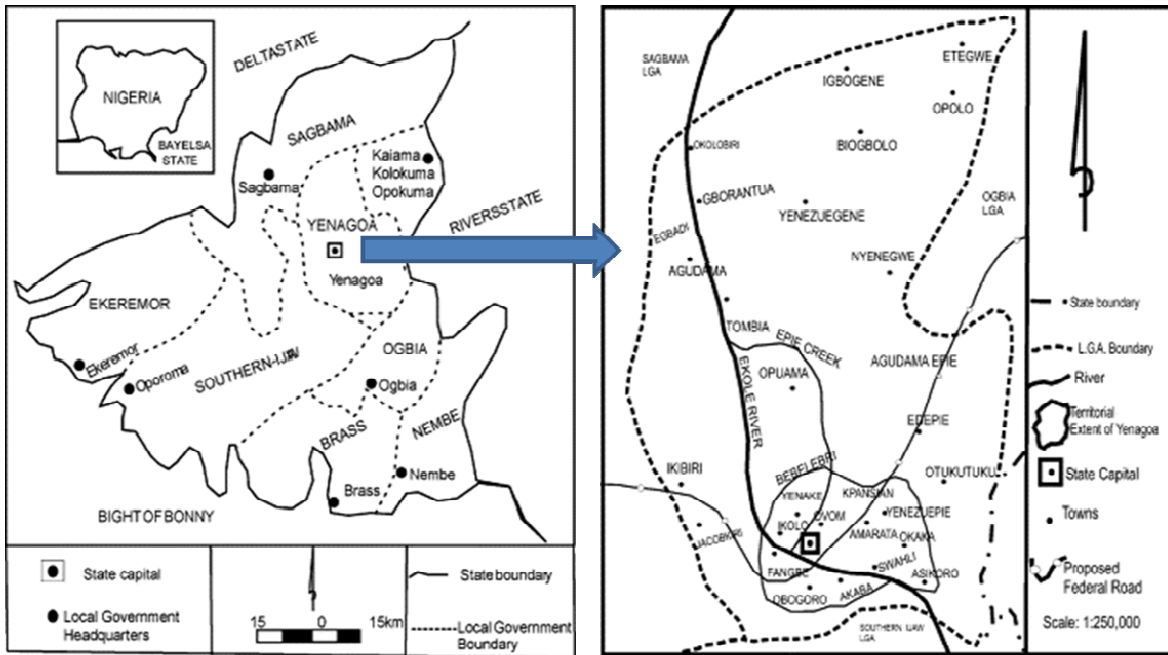
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**Fig. 1. Map showing sample sites.**  
**Left: Map of Bayelsa State, Nigeria, showing the different Local Government Areas (LGA)**  
**Right: Map of Yenagoa LGA showing the sample sites (Swali, Opolo and Edepie)**  
**(Source: National Population Commission Yenagoa, 1996)**



**Fig. 2: Irvingia nuts displayed for sale in a typical market in Yenagoa metropolis of Bayelsa State, Nigeria**

Table 1: Comparative fungal population and quality, of *Irvingia* kernels obtained from different markets in Yenagoa metropolis of Bayelsa State, Nigeria

Market	Fungal population		Fungal species isolated
	Mean Log CFU	Weighted CFU	
Swali	5.07 <sup>a</sup>	1.17E+05	<i>Rhizopus</i> sp and <i>Mucor</i> sp.
Opolo	5.38 <sup>b</sup>	2.40E+05	<i>Rhizopus</i> sp, <i>Mucor</i> sp., <i>penicillium</i> sp and <i>Candida</i> sp
Edepiye	5.84 <sup>c</sup>	6.92E+05	<i>Rhizopus</i> sp, <i>Mucor</i> sp and <i>Aspergillus</i> sp.

Table 2: Relative amounts of different phytochemicals extracted from *Irvingia* nuts bought from different markets in Yenagoa metropolis, Bayelsa State, Nigeria

Market	Relative mounts of Phytochemicals				
	Alkaloid	Flavonoid	Saponnin	Tannin	Glycosides
Swali	+	+++	++	++	-
Opolo	++	+++	++	++	-
Edepiye	++	+++	++	++	-

- Phytochemical not detected

+ Low intensity of precipitate/colour indicating presence of phytochemical

++ Moderate intensity of precipitate/colour indicating presence of phytochemical

+++ High intensity to of precipitate/colour indicating presence of phytochemical

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