Pathological Evaluation and Biochemical Analysis of Coated and Decoated Seeds of *Irvingia gabonensis*

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Abstract
Laboratory Studies on the post-harvest pathology and proximate composition of coated and decoated seeds of lrvingia gabonensis were carried out in the Plant pathology laboratory of Rivers State University, Port Harcourt. Results showed that decoated seeds harboured more fungi and higher degrees of incidence than coated seeds. Different fungi with varying degrees of incidence were isolated. Generally, four genera of fungi (Penicillium, Aspergillus, Botyriodiplodia and Rhizopus) were isolated from post-harvest l. gabonensis seeds. Aspergillus was more predominant than other fungi species isolated. The percentage incidence of fungi isolated from coated l. gabonensis ranged from Rhizopus stolonifer 63%, Aspergillus niger 36% Aspergillus flavus 20%, and Botyriodiplodia theobromae 10%. While from decoated samples R. stolonifer 80%, A. niger 43%, A. nidulans 15%, A. flavus 30%, Penicillium italicum 8.5% and A. tamari 20% were isolated. All the fungal isolates were found to be pathogenic to fresh healthy seeds of l. gabonensis causing general soft rot. Result also revealed that the proximate values comprising of moisture, lipid and carbohydrate were higher in decoated seeds, while Ash, fibre and protein were higher in coated samples. Mineral content comprising of calcium, Phosphorus, Sodium, Potassium, Magnesium and Iron were higher in coated seeds.

Key words: *Coated, decoated, lrvingia gabonensis, fungi, proximate composition.*

I. INTRODUCTION

lrvingia gabonensis (Aubry-Lecomte ex O'Rorke) is a highly, economically important tree native to most tropical forests in West and central Africa (Lowe *et al*., 2002). *lrvingia gabonensis* is sometimes called bush mango or African mango because the trees bear mango-like fruit (Matos *et al*., 2009).

The kernel of the seeds, 3cm in diameter, is enclosed in a fibrous testa, and is especially valued for their fat and protein rich nuts which serves as a sauce thickening agent and oil (Matos *et al., 2009).* The seeds are high in fat, similar to other nuts and seeds and contain an extraordinary fibre content of 14% (Giami *et al,* 1994).
The humid lowlands of Cameroun, Nigeria and Cote d'lvoire have been identified as the major sources of *lrvingia gabonensis* kernels in local and international trade (Ayuk *et al.,*

1999). The demand for bush mango kernels has resulted in excessive exploitation in the bush at such a rate that the sustainability of these natural resourced has been the concern of various workers (NRC 1991).

In addition to its nutritional and economic benefits *lrvingia gabonensis* is highly valued for its health and medical benefits Studies have shown that seed extract of *lrvingia gabonensis* caused a significant reduction in body weight among obese people in Cameroon (Ngondi *et al.*, 2005).

Post-harvest diseases are responsible for heavy losses of agricultural produce during storage, reduce food quality and render them unfit for human consumption (Doyle 2007). Generally, microorganisms are adjudged the most notorious culprits, amongst other factors, responsible for postharvest diseases of crops (Ray *et al*., 2000) and fungi in particular, have been found to be one of the principal causes of postharvest losses in many zones of the world, ranking alongside insects and pest. The occurrence of fungi in food does not only render the food undesirable in terms of palatability but consumers of fungal infected food stand the risk of huge health hazards. This is because some fungi implicated with postharvest diseases are able to produce mycotoxins known to be highly toxic, carcinogenic, and are able to also suppress one's immune system, cause growth retardation, liver disease, and death of both humans and domestic animals Bankole *et al.*, 2005.

Recent studies have shown that post-harvest commercial *lrvingia gabonensis* are laden with several fungal species (Etebu and Bawo 2012a), some of which are known to produce mycotoxins. Although, there is yet no report of illness arising from consumption of *lrvingia* kernels, other workers have demonstrated the occurrence of mycotoxins among *lrvingia* kernels in storage (Adebayo-Tayo *et al.*, 2006), making their consumption a potential health risk.

Similarly, recent studies have shown that *lrvingia gabonensis* seeds possess appreciable amount of proximate compositions (Sanyaolu *et al.*, 2014). Although post-harvest mycodeterioration on the proximate compositions of *lrvingia gabonensis* seeds have been reported (Sanyaolu *et al.*, 2014), there is dearth of studies on the proximate composition of coated and decoated seeds of *lrvingia gabonensis*. It is against this backdrop, that this research was carried out. Hence in this research, a comparative study on spoilage rot of decoated and coated seeds of *lrvingia gabonensis* was done and their proximate composition investigated.

Findings from this work would avail us the prerequisite information to assess mycoflora associated with coated and decoated seeds of *lrvingia gabonensis*, as well as the proximate compositions for beneficial use.

II. MATERIALS AND METHODS

Collection of Samples

lrvingia gabonensis kernels (coated and decoated) were purchased from Rumukwurushi Market in Port Harcourt, Nigeria. The samples were taken to the plant pathology laboratory for further studies.

Proximate Composition Determination

The samples of *lrvingia gabonensis* were taken to the laboratory for the determination of their proximate compositions comprising of ash, moisture, fibre, lipid, carbohydrate and protein, as

well as their mineral content. These parameters were determined according to the method of Association of Official Analytical Chemist (AOAC, 1990).

Media Preparation

The medium used for fungal isolation was the Sabouroud Dextrose Agar (SDA). This was prepared by weighing 32.8g of Sabouroud Dextrose Agar (SDA) into a 500ml conical flask, Distilled water (500ml) was added into the flask with a measuring cylinder and stirred to homogenize. The mouth of the conical flask was plugged with sterile cotton wool and wrapped with foil. The conical flask with its contents was autoclaved for 15 minutes at 121^{o}C at 1.1kg cm^{-3} pressure. Sterile petri dishes were prepared and the mixture dispensed into them while still hot and allowed to solidify.

MYCOLOGICAL STUDIES

Isolation and identification of fungi

Five kernels of *lrvingia gabonensis* used were washed in tap water, rinsed in distilled water and surface sterilized with 5% sodium hypochlorite for 5 minutes and rinsed twice in sterilized distilled water after which they were aseptically introduced into the SDA in petri dishes equidistantly, in triplicate.

The inoculated plates and their contents were incubated for 7 days at room temperature of 25$\pm$3^{o}C for five days. Pure cultures of fungi growing in mixtures were obtained thereafter. Pure cultures of the isolates were made after series of isolation. The fungi were later identified based on colour, spore morphology and the nature of the mycelia according to the key of Olds (1983).

Percentage incidence of fungi

Incidence of fungi was determined by using the formula:

$$\frac{Total\ number\ of\ occurence\ of\ a\ particular\ fungi}{Total\ number\ plated\ sample} \times \frac{100}{1}$$

Pathogenicity Studies

Pathogenicity test was carried out to determine if the fungal isolates responsible for the spoilage of *lrvingia gabonensis* kernels were capable of causing rot of healthy fresh samples. The procedure described by Agrios (2005) and (Trigiano *et al.*, 2004) was used. Healthy kernels of *lrvingia gabonensis* were washed in tap water surfaced sterilized with 5% sodium hypochlorite and rinsed twice in sterile distilled water.

Each of the fungal isolate was aseptically transferred onto the healthy *lrvingia gabonensis* kernels on damp blotter papers in petri dishes and incubated at room temperature of 25$\pm$3^{o}C for 5 days. Petri dishes containing kernels of *lrvingia gabonensis* samples without the fungal isolates served as control. Data generated from fungal isolates and proximate analysis were interpreted using percentages and standard error.

III. RESULTS AND DISCUSSION

The results of the proximate composition and mineral content of the seeds of *lrvingia gabonensis* (coated and decoated) are presented in Table 1 and 2 respectively.

Table 1 Proximate Composition and Mineral Content of Coated *lrvingia gabonensis*.

S/N	Parameter	Values %
1.	Moisture	11.52 ± 0.24
2.	Ash	6.5 ± 0.24
3.	Fibre	7.2 ± 0.20
4.	Lipid	6.8 ± 0.20
5.	Carbohydrate	41.2 ± 0.20
6.	Protein	26.78 ± 0.30
7.	Calcium	1.3 ± 0.30
8.	Phosphorus	3.6 ± 0.24
9.	Sodium	0.50 ± 0.00
10.	Potassium	5.6 ± 0.24
11.	Iron	0.04 ± 0.00
12.	Magnesium	0.31 ± 0.00
13.	Tannin	0.85 ± 0.10
14.	Viscosity	35cps ± 0.43

Table 2 Proximate Composition and Mineral Content of Decoated *lrvingia gabonensis*

S/N	Parameter	Values %
1.	Moisture	13.5 ± 0.22
2.	Ash	4.5 ± 0.22
3.	Fibre	2.05 ± 0.10
4.	Lipid	10.4 ± 0.18
5.	Carbohydrate	49.41 ± 0.55
6.	Protein	20.13 ± 0.20
7.	Calcium	1.2 ± 0.20
8.	Phosphorus	3.4 ± 0.33
9.	Sodium	0.45 ± 0.00
10.	Potassium	5.5 ± 0.24
11.	Iron	0.03 ± 0.00
12.	Magnesium	0.32 ± 0.00
13.	Tannin	0.48 ± 0.00
14.	Viscosity	38cps ± 0.41

Coated *lrvingia gabonensis* recorded the following values;
Moisture 11.52, Ash 6.5, Fibre 7.2, Lipid 6.8, Carbohydrate 41.2 and protein 26.78. The protein content of coated *lrvingia gabonensis* was higher than that of decoated *lrvingia gabonensis*. Generally, coated *lrvingia gabonensis* recorded higher values of Ash, fibre and protein when compared to decoated samples. While decoated samples had higher values of moisture, lipids and carbohydrates. Decoated *lrvingia gabonensis* recorded the following values: Moisture 13.5, Ash 4.5, Fibre 2.05, Lipid 10.4, Carbohydrate 49.41 and protein 20.13. The mineral content value of coated samples of *Irvingia gabonensis* was higher in K

(potassium) 5.6, followed by P (phosphorus) 3.6, Calcium 1.3, Sodium 0.50, Magnesium 0.31 and the least was Iron 0.04.

For decoated samples, the following values of mineral content were recorded. Potassium 5.5 took the lead, followed by phosphorus 3.4, calcium 1.2, sodium 0.45, magnesium 0.32 and the least Iron 0.03. The range of values took the same order as the coated samples. The values of the mineral content were higher in coated samples than decoated samples except magnesium. The viscosity was higher in decoated samples 38cps compared to coated samples 35cps. The phytochemicals; Tannin oxalate and HCN in coated samples were 0.85, 1.32 and 0.1 respectively, while those of decoated samples were 0.48, 1.11 and 0.05 respectively. In general, the values of phytochemicals of coated samples were higher than decoated samples.
The results of the fungal isolates from coated and decoated seeds of *lrvingia gabonensis* are presented in table 3.

Table 2: Mean percentage incidence of fungi isolated from seeds (coated and decoated of *lrvingia gabonensis*

Fungal isolates	**Coated *l. gabonensis***	**Decoated *I. gabonensis***
Rhizopus stolonifer	63 ± 0.45	80 ± 0.45
Aspergillus niger	36 ± 0.40	43 ± 0.60
Aspergillus flavus	20 ± 0.27	30 ± 0.33
Aspergillus nidulans	-	15 ± 0.33
Penicillium italicum	-	8.5 ± 0.29
B. theobromae	10 ± 0.58	-

In decoated samples of *l. gabonensis*, *Rhizopus stolonifer (80%)* had the highest incidence followed by *Aspergillus niger* (43%), *Aspergillus flavus* (30%), *Aspergillus nidulans* (15%) and *Penicillium italicum* (8.5%). It followed the same order in coated samples; 63%, 36%, 20% and 10% for *R. stolonifer, A. niger, A. flavus* and *B. theobromae* respectively. Comparing coated and decoated samples, decoated samples of *I. gabonensis* harboured the highest number and percentage incidence of all the fungi isolated except *B. theobromae* (10%) which occurred only in coated samples.
Rhizopus stolonifer, Aspergillus niger and *Aspergillus flavus* were dominant in both coated and decoated *I. gabonensis,* but higer in decoated samples.
Aspergillus nidulans and *Penicillium italicum* occurred only in decoated samples.

Generally, microorganisms are adjudged the most notorious culprits, amongst other factors, responsible for postharvest diseases of crops (Ray *et al.*, 2000). These micro-organisms, particularly fungi bring about heavy losses of agricultural produce during storage, reduce food quality and render them unfit for human consumption (Doyle, 2007). Similar fungi isolated from this study have been reported earlier by other workers. Etebu and Bawo (2012a) have shown that postharvest commercial *lrvingia* kernels are laden with several fungal species, some of which are known to produce mycotoxins.

Apparently, decoated samples of *lrvingia gabonensis* had a higher incidence of fungi compared to coated samples. This could be attributed to the method of handling and processing. It has been reported that the method of processing, handling and preservation affects the level of contamination and influence the microbial load of agricultural products. (Chukwu *et al.,* 2009). All the fungal isolates from decoated and coated seeds of *lrvingia*

gabonensis were found to be pathogenic causing general soft rot of the seeds leading to the decay of the seeds and loss of quality. The fungi re-isolated from *lrvingia gabonensis* seeds were the same as the initial isolates that were introduced confirming the fungi to be associated with the rot of seeds of *lrvingia gabonensis*. The isolation of less fungal loads from coated samples of *lrvingia gabonensis* is because seeds are generally protected by differentiated integumentary structures which serve as barriers to microbial invasion.
Four genera of fungi (*Aspergillus, penicilium, Rhizopus* and *Botrytis spp*) were isolated from postharvest *lrvingia gabonensis* seeds studies in this work.

This agrees with earlier works by Etebu (2012) who revealed *Aspergillus*, *Botrytis*, *Penicillium* and *Mucor* have been associated with post-harvest *lrvingia gabonensis* fruits. This result is also comparable to the findings of Etebu and Bawo (2012a).
On the proximate composition and phytochemicals, coated and decoated seeds of *lrvingia gabonensis* recorded 0.85 and 0.48% of tannin respectively. Tannis have been shown to be very significant roles in human medicine and treatment of ailments Addae-Mensah, 1992). They are astringent bitter plant polyphenols; known to complex with leaf proteins, and coenzymes from soil microorganisms, and in so doing, adversely affect nitrogen availability (Kraus *et al.,* 2004; Nierop *et al.,* 2006). The fibre content of coated and decoated seeds of *I. gabonensis* were 7.2 and 2.05% respectively. This observation however, negates the assertion of Giami *et al.,* (1994) who reported a fibre content of 14%.

This study further revealed that *I. gabonensis* contains an appreciable amount of protein; 26.78 and 20.13% from coated and decoated seeds respectively. This agrees with the findings of Matos *et al.,* (2009) who reported that the seed is rich in protein.
They were also found to be comparable to 26.80 – 29.20% reported for *Canavalia glidata* by Barminas *et al.,* (Barminas *et al., 2004).* These values give the seeds positive attributes, as plant proteins are scarce and this protein content can furnish the essential amino acid needed for healthy growth and repair of tissues (Igwenyi, 2008).

The carbohydrate content 41.2 – 49.41% were lower than 69.70% for *I. gabonensis* reported by Eddy and Udoh, (2005). This property of carbohydrates is in line with the work of Ejiofor who explained that flour of *Irvingia gabonensis* are still acceptable in terms of its colour, taste, texture and drawability after a period of time in ambient conditions, and is more viscous, with greater emulsifying properties than undefatted flour (Ejiofor, 1994).
The lipid content was low; 6.8 -10.4% for coated and decoated samples respectively. The result on lipid composition showed that *I. gabonensis* seed is not an oil seed and cannot serve as a commercial source of vegetable oil. The values were however comparable to 8.30% in Mangifera Indica (Eddy and Udoh, 2005).

IV. CONCLUSION

This research has shown that coated seeds of commercial *I. gabonensis* harbored less fungi than decoated seeds. Four genera of fungi (*Aspergillus, Penicillium, Rhizopus and Botyriodiplodia)* were isolated from *I. gabonensis* seeds studied in this work. The seed do contain appreciable amount of carbohydrate, protein and viscosity, hence it serves as both soup thickener and food condiment. Findings from this work avail us the prerequisite information on the pathology of commercial *I. gabonensis* seeds and its derivable potential benefits.

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