Biochemical Evaluation and Fungi Pathogens Associated with Fresh and Boiled *Detarium microcarpum* (Ofor) in Rivers State, Nigeria

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Abstract
Comparative studies on the fungi pathogens associated with fresh and boiled Detarium microparpum as well as their biochemical analysis were done in the plant pathology laboratory of Rivers State University, Port Harcourt; Results showed that boiled samples of D. microcarpum harboured more fungi species and higher degrees of incidence than fresh samples. Five genera of fungi (Rhizopus, Aspergillus, Botyriodiplodia, Fusarium and Penicillium were isolated from fresh and boiled seeds of commercial D. microcarpum. Aspergillus was more predominant than other fungi species isolated. The percent incidence of fungi isolated from fresh D. microcarpum ranged from Rhizopus stolonifer 60%, Aspergillus niger 68%, Aspergillus flavus 56%, Rhizopus stolonifer 50%, Fusarium oxysporum 30%, Penicillium digitatum 10.5% and Botyriodiplolia theobromae 6% were isolated.
All the fungal isolates were found to be pathogenic to healthy seeds of D. microcarpum. Results also revealed that proximate values comprising of Carbohydrate, Protein, were higher in fresh samples while Moisture content was higher in boiled samples. There was no significant difference in the values of Ash, Fibre and Lipid in the fresh and boiled samples. Similarly, there was no significant difference in the values of Mineral content comprising of Calcium, Phosphorus, Sodium, Potassium, Magnesium and Iron among fresh and boiled samples. Viscosity had the same value in both samples.

Key words: *Detarium microcarpum, fresh, boiled, fungi, proximate composition.*

Introduction

Detarium microcarpum is a member of the subfamily Caesalpinaceae and Fabaceae family (Abdalbasit *et al.*, 2009) that thrives in both the Rainforest and Savanna zones in Nigeria. It is known by various names as “Ofor” (Igbo), “Taura” (Hausa) and “Ogbogbo” (Yoruba) in Nigeria.

The plant is a tree of up to 10m high with twisted trunk and spreading crooked branches. The fruits are drupe like, circular or disc-shaped with a tangled network of fibres. The seeds occur singly and are within the hard disc shaped, brownish shell. The fruits are eaten raw or cooked. It is used to emulsify, flavor and as thickening agents used to thicken soup, bake cakes and bread, added when producing baby food and used to prepare local beer. The leaves are used as condiment or vegetables, as are its flowers (Kouyate A.M and Van D.P 2006).

The seeds yield 7.5% oil, with linoleic acid being the predominant fatty acid. The gum content (water-soluble polysaccharides) is high. The hulled seed flour contains per 100g: water 3.5 – 6.5g, crude fibre 3g, crude fat 13-15g, crude protein 13.5-27g, carbohydrate 39g, Ca 500mg, Mg 500mg, Fe 100 mg. The major alcohol-soluble sugar in the hulled seed flour is sucrose. The seed flour is used as a traditional emulsifying, flavouring and thickening agent, Roasting the seeds increases crude fat content, crude protein content, ash content, the water absorption capacity, oil absorption capacity and gelation temperature, but decreases carbohydrate content, crude fibre content, the emulsion capacity and the swelling index (The free encyclopedia 2012).

The barks, leaves and roots are prepared as decoctions to treat rheumatism, venereal diseases, urogenital infections, haemorrhoids, stomach ache, intestinal worms and diarrhea including dysentery. They are also used against malaria, leprosy and impotence.

A decoction of the powered bark is widely taken to alleviate pain, e.g. headache, sore throat, back pain and painful menstruation. The fresh bark or leaves are applied to wounds, to prevent and cure infections. They are also used to treat fainting, convulsions, constipation, measles, hypertension, itch and tiredness. In Senegal, a mixture of the leaves pounded in milk is considered very efficient for snakebites. The heated roots are sweet scented and are used as a perfume by Dinka women in Sudan, and as a mosquito-repellent in Chad (Kouyate, A.M. and Van, D.P., (2006).

Some fungi could be mere surface contaminants while others could be deep seated, surviving long enough to cause considerable damage both to the seed quality and the consumer. Many of these surface contaminants include fungi which have been isolated from seeds, fruits, vegetables and stored products in the tropics particularly in Niger Delta and are reported to produce mycotoxins in high concentrations.
Recent studies have shown that post-harvest commercial *Detarium microcarpum* seeds are laden with several fungi species (Ikechi-Nwogu and Elenwo 2012).

Aspergillus, *Rhizopus, Fusarium*, *Botrydiplodia* and *penicillium spp* have been associated with post-harvest *Detarium microcarpum* (Ikechi-Nwogu and chime (2007).
Despite, the numerous advantages this crop confers on the general populace, a little research has been done on the seed, particularly on post-harvest pathology and proximate analysis of the fresh and boiled seeds of *D. microcarpum.* This observation informed the decision to carry out this research. Hence, the research is aimed at investing the fungal species associated with fresh and boiled seeds of *D. microcarpum*, as well as the proximate compositions for medicinal and beneficial use.

Materials and Methods
Collection of samples
Fresh and boiled *D. microcarpum (*Ofor) seeds were purchased from oil mill market in Port Harcourt, Rivers State, Nigeria.
The samples were taken to the Plant Pathology laboratory for further studies.

Proximate composition determination
The samples of Ofor 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 Chemists (AOAC, 1990).

Media Preparation

The medium used for fungal isolation was the Sabouraud Dextrose Agar (SDA). This was prepared by weighing 32.8g of Sabouraoud 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 seeds of *D. microcarpum* used were washed in tap water, rinsed in distilled water and surface sterilized with 5% Sodium hypochlorite 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 28 ± 2^{o}C. Pure culture of fungi growing in mixtures was 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).

Pathogenicity Studies

Healthy samples of *D. microcarpum* were washed in tap water and surface sterilized in 5% sodium hypochlorite. The fungi isolates were aseptically inoculated onto the healthy seeds on damp blotter papers in petri dishes and incubated at room temperature of 28 ± 2^{o}C for five days. Petri dishes containing seeds of *D. microcarpum* without the fungal isolates served as control. The extent of rot was determined using the method as described by Agrios (2005) and Trigiano *et al.*, 2004.

Mean percentage incidence of fungi

The mean percentage incidence of fungi was calculated using the formula:

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

Results and Discussion

The results of the proximate composition of fresh and boiled seeds of *D. microcarpum* are presented in Table 1 and 2 respectively. The usual constituents of food determined by proximate analysis include Protein, Carbohydrate, Crude Fibre, (roughage), Lipid (fat and oil) Ash, Moisture (water) contents.

The results of the proximate composition of fresh *D. microcarpum* seeds as shown in Table 1 were moisture 12.5, Ash 3.2, Fibre 3.5, Lipid 14.2, Carbohydrate 48.0, Protein 18.6, Calcium 1.15, Phosphorus 2.7, Sodium 0.22, Potassium 3.5, Iron 0.02 and Magnesium 1.05, Viscosity 22.5cps.

For boiled samples, the following values were recorded. Moisture 18.5, Ash 3.0, Fibre 3.2, Lipid 14.5, Carbohydrate 45.0, Protein 15.8, Calcium 1.16, Phosphorus 2.6, Sodium 0.24, Potassium 3.6, Iron 0.02, Magnesium 1.04, Viscosity 22.5cps. (Table 2)

Fresh seed of *D. microcarpum* recorded higher values of Ash, Fibre, Carbohydrate, Protein, Phosphorus, and Magnesium, while boiled seeds were higher in Moisture, Lipid, Calcium, Sodium and Potassium. However, the values of viscosity and iron were the same in both samples. (Table 2)

The results of the fungal isolates from fresh and boiled seeds of *D. microcarpum* are presented in table 3.

Table 1: Proximate Analysis of Fresh *D. microcarpum* Seeds

Parameter	Values %
Moisture	12.5 ± 0.24
Ash	3.2 ± 0.20
Fibre	3.5 ± 0.24
Lipid	14.2 ± 0.18
Carbohydrate	48.0 ± 0.55
Protein	18.6 ± 0.22
Calcium	1.15 ± 0.30
Phosphorus	2.7 ± 0.10
Sodium	0.22 ± 0.00
Potassium	3.5 ± 0.24
Iron	0.02 ± 0.00
Magnesium	1.05 ± 0.20
Viscosity	22.5cps ± 0.50

Table 2: Proximate Analysis of Boiled *D. microcarpum* Seeds.

Parameter	Values %
Moisture	18.5 ± 0.24
Ash	3.0 ± 0.20
Fibre	3.2 ± 0.22
Lipid	14.5 ± 0.55
Carbohydrate	45.0 ± 0.40
Protein	15.8 ± 0.18
Calcium	1.16 ± 0.20
Phosphorus	2.6 ± 0.24
Sodium	0.24 ± 0.10
Potassium	3.6 ± 0.24
Iron	0.02 ± 0.00
Magnesium	1.04 ± 0.30
Viscosity	22.5cps ± 0.50

Table 3: Mean percentage incidence of fungi isolated from fresh and boiled seeds of *D. microcarpum* Seeds.

Fungal Isolates	Fresh *D. microcarpum*	Boiled *D. microcarpum*
Rhizopus stolonifer	60 ± 0.80	50 ± 0.58
Aspergillus niger	45 ± 0.22	68 ± 0.90
Aspergillus flavus	42.85 ± 0.50	56 ± 1.20
Botyriodiplodia theobromae	-	6 ± 0.32
Fusarium oxysporum	-	30 ± 0.26
Penicillium digitalum	-	10.5 ± 0.24

From the results obtained *Rhizopus stolonifer* (50%), *Aspergillus niger* (68%), *Aspergillus flavus (56%), Botyriodiplodia theobromae (6%), Fusarium oxysporum (30%), Penicillium digitalum (10.5%)* were isolated from boiled seeds. While *Rhizopus* (60%), *Aspergillus niger (45%),* and *Aspergillus flavus (42.85%)* were isolated from fresh seeds.

Rhizopus stolonifer, Aspergillus niger, and *Aspergillus flavus* were predominant in *D. microcarpum.* Comparing fungi contamination of boiled and fresh seeds, it showed that fungi obtained from boiled samples had the highest percentage incidence of fungi compared to fresh seeds. (Table 3)

Aspergillus niger, had the highest occurrence followed by *Rhizopus stolonifer*, *Aspergillus flavus, Fusarium oxysporum, Penicillium digitarium* and *Botyriodiplodia theobromae.*

Rhizopus and *Aspergillus* species were dominant in the two samples (fresh and boiled) while *Botyriodiplodia theobromae*, *Fusarium oxysporum* and *Penicillium digitarium* were found only in boiled sample.

The boiled samples were found to have a higher level of percentage incidence of fungi due to the fact that they were exposed to fungal activity than the fresh seeds. The boiled seeds absorbed moisture, fungi thrive in moisture. Thus, the boiled form is more prone to fungal attack. Moreso, the method of processing, handling and preservation affects the level of contamination and influence the microbial load of agricultural products (Chukwu *et al., 2009)*

The climatic conditions prevalent in an open market had been reported to favour the survival of some fungi also isolated from other fruits, vegetables, food spices and stimulants (Elenwo, 1996).

The result from this study agrees with Ikechi-Nwogu and Chime (2017) who found *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium* and *Botyriodiplodia* spp on seeds of *Detarium microcarpum.* The fungal species isolated is also comparable to the results of earlier workers who isolated *Aspergillus*, *Fusarium*, *Botyriodiplodia*, *Rhizopus*, *Penicillium* and Mucor spp from seeds sold at the open markets. (Ibrahim 2014, Liamngee *et al., 2016).*

On the proximate analysis the result revealed that boiling the seeds of *D. microcarpum* increased the moisture content, which is not unexpected because seeds absorb water when boiled, but boiling decreased carbohydrate and protein content (Table 2). It thus suggests that protein and carbohydrate depreciate with heat.

There was no significant difference in the values of fibre, ash and lipid, when compared between boiled and fresh seed samples. This applies also to the values of the mineral content; calcium, phosphorus, potassium, sodium, iron and magnesium. The protein contents of the fresh and dry samples 18.6% and 15.8% were higher than 12.19% reported by Igwenyi and Azoro (2014) on *D. microcarpum* from the open market. *D. microcarpum* seeds have not been reported as good sources of protein. The carbohydrate composition 48% and 45% for fresh and boiled seeds respectively were significantly lower than the report of Igwenyi and Akubugwo (2010). The value was also significantly lower than 70.38% reported by Igwenyi and Azoro (2014) on the same seed. The decrease could be as a result of the processing method in the preparation of the seed samples and other environmental factors. The carbohydrate contents were comparable to 57-59% reported for Brachystegia *eurycoma* and *Detarium microcarpum* (Uhegbu *et al.*, 2009).

The percentage compositions of lipids in the samples were 14.2 and 14.5% for fresh and boiled samples respectively (Table 1 and 2). These values were higher than 7.41% reported by Igwenyi and Azoro (2014); Igwenyi and Akubugwo (2010). The results were comparable to 14.0 – 18.5% reported by Uhegbu *et al.*, (2009). These variations in the oil contents may be attributed to differences in climatic conditions, soil properties, average rainfall, freshness and storage conditions/time of the seeds.

The analysis of lipid contents showed that the seed of *D. microcarpum* is not an oil seed or oil crop and cannot serve as commercial source of vegetable oil. The value of proximate lipid composition was also comparable to 15% reported in *Detarium microcapum* by Akpata and Miachi (2001).

The percentage fibre was 3.5 and 3.2% for fresh and boiled samples respectively. These values are comparable to the values for *D. microcapum* (2.90%) reported by Akpata and Miachi (2001) respectively as well as the report of (Igwenyi and Azoro, 2014).

Fibre regulates bowel actions and may help to guard against colon and rectal cancer as well as in diabetes. Fibre shortens the transit time of food through the gastrointestinal tracts, reduces low density lipoprotein and hence keeps the gut healthy. Fibre supplements or fibre-rich foods may functions as normal dietary agents by modulating the digestive and absorptive process (Okaka *et al.*, 2006).

The ash contents values were low with fresh seed having a value of 3.2% while boiled sample had a value of 3.0%. This agrees with the assertion of Igwenyi and Azoro (2014) who reported 3.09% Ash for fresh seed of *D. microcapum*.

The percentage moisture contents were 12.5 and 18.5 for fresh and boiled samples respectively. These values were significantly higher than the report of Igwenyi and Azoro (2014) on fresh samples of the same seed sold in the open market. The variations in proximate contents could be attributed to climatic conditions, soil fertility and soil type. Generally, the results of the proximate compositions were similarly comparable to protein 19.69 – 39.08%, carbohydrate 32.78 – 67.26%, lipid 2.70 – 21.08% and fibre 1.78 – 4.68% reported by Okwu and Orji (2007).

Conclusion

D. microcapum seed has an appreciable percentage yield of carbohydrate that serves both as thickener and fuel source for the generation of energy currency of the cell. The protein contents showed that they can provide the amino acids needed to support the metabolic activities of the body. However, the decrease in carbohydrate and protein contents of boiled sample showed that boiling reduces certain nutrients of plant foods. Boiled samples harboured more fungi species and percentage incidence than fresh samples as revealed from this work. It therefore suggests that boiled seeds of *D. microcapum* sold in the open market is a good substrate for the growth of pathogenic fungi, most of which are known to produce mycotoxin which in turn is detrimental to human health because of the associated diseases.
There is therefore a need to increase public health awareness and to develop suitable management practices of food condiments in order to improve food security and safeguard the health of the consumers.

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