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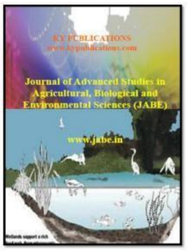
EFFECT OF BOILING ON THE PATHOLOGY AND BIOCHEMICAL PROPERTIES OF *Mucuna sloanei* SEEDS

Emiri,U. N.[1] Chukwu, E. C[2]
[1]Department of Agricultural Education, Isaac Jasper Boro College of Education, Sagbama, Bayelsa State, Nigeria.
[2]Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria.
Corresponding Email: ucheemiri@gmail.com



ABSTRACT
There is dearth of research on the post-harvest pathology and biochemical properties of *Mucuna sloanei* seeds. Hence, in this research, effect of boiling on the pathology and biochemical properties of *Mucuna sloanei* seeds is investigated. Results showed that boiled samples of *M. sloanei* had a higher percentage incidence of fungi than raw samples.Five genera of fungi (*Rhizopus, Aspergillus, Fusarium, Penicillium and Alternaria*) were isolated from raw and boiled seeds of *M.sloanei*. *Aspergillus* and *Rhizopus* were predominant in both samples. The percentage incidence of fungi isolated from raw samples ranged from *Aspergillus niger 50%, Rhizopus stolonifer 48%, Aspergillus flavus 32.8%*. While from boiled samples, *Fusarium solani 80%, Aspergillus niger 60%, Rhizopus stolonifer 52%, Aspergillus flavus 30%, Penicillium italicum 20% and Alternaria altermata 12.5%* were isolated.All fungal isolates were found to be pathogenic to raw and boiled healthy seeds of *M. sloanei*. On the proximate composition, results also revealed that boiling decreased the contents of ash (3.3-3.2%), fiber (3.8-3.3%), lipid (8.3-8.1%) and carbohydrate (54.5-49.6%) but increased the moisture (10.5-14.6%) and protein (19.6-21.2%) contents. Boiling also affected the mineral contents comprising of Calcium, Phosphorus, Potassium, Magnesium andIron.
Key Words:*Mucuna sloanei* seeds, proximate composition, mineral content, Raw, Boiled, Fungi.

1. INTRODUCTION

Mucuna is a genus of about one hundred (100) accepted species of climbing vines and shrubs of the family *fabaceae*, found worldwide in the wood lands of tropical areas (Bressani R. 2002). Like other legumes, *Mucuna* plants bear pods. The seed pods are covered with microscopic velvety hairs (called *trichomes*) that can be extremely painful if they get into your eyes or could cause itchy blister when they come in contact with the skin. They are generally bat pollinated and produce seeds that are buoyant sea beans. They have a characteristics three-layered appearance, appearing like the eyes of a large mammal in some species and like a hamburger in others (Most notably *Mucuna sloanei*).

*Mucuna sloanei*is used by the Igbo Community in Sub-Sahara Africa as condiment or part of the main dish. (Ukachukwu *et al.,* 2002).Seeds of *Mucuna sloanei* popularly called Ukpor are used as soup thickener and Vegetable oil, Beverages and food items (Wayekeche *et al.,* 2003). Its seeds are cracked by hitting with a hard object before cooking, then dehulled, ground, mixed with red oil palm to obtain yellow powder and marketed as soup thickener. All parts of *Mucuna* plant are reported to possess phytochemicals of high medicinal value and veterinary importance and also constitute as an important raw material in Ayurvedic and folk medicines.

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Hydrothermal treatments, fermentation and germination have been shown to be most effective in reducing the anti-nutrients of the seeds. Several anti-nutritional compounds of the seeds serve in health care and considerable attention has been drawn towards their antioxidant properties and potential health benefits. (Adebowale *et al.,* 2005).It has nutritional potential as a rich source of protein (23 – 35%) (Bressani 2002).
The functional properties of seed flour assume importance in the development of food product. Proteins and starch are the main contributors for changes in functional properties such as Bulk density, oil and water absorption and least gelatin concentration.

The seeds of *Mucuna sloanei*possess good functional properties and in Vitro protein digestibility. (Adebowale *et al.,* 2005)

Beside typical medicinal properties, several phytochemicals of *Mucuna* seeds serve in health care in a variety of ways. The phytic acid of *Mucuna sloanei*possess antioxidant, anti-carcinogenic and hypoglycemic activities (Graft and Eaton 1990; Richard and Thompson 1997) and are effective at low concentrations. Saponins are recently shown to have hypocholestrolemic as well as anti-carcinogenic effect (Koratkar and Rao 1997). Tannins are also known to possess health benefits, where they are 15 – 30 times more efficient in free radical quenching activity than Trolex and other simple Phenolics (Hurrel et al; 1999)
The plant pathogenic fungus *Mycosphaerella Mucunae* is named for being first discovered on *Mucuna.*Fungal and Mycotoxin contamination is also of main concern to minimize the economic losses and reduce the potential health risks to humans and livestock (Ueno 2000). The seeds of *Mucuna sloanei*are susceptible to fungal and mycotoxin contamination. Obiakor-Okeke *et al.,*(2014)isolated *Aspergillus* and *Rhizopus* species from cooked *Mucuna sloanei*seeds. Gbarabe *et al*.,(2014) isolated *Aspergillus*, *Penicillium* and *Botryodiplodia* species from the same seed.

MucunaSloanei is one of the underutilized food crops in Nigeria. Most often, Cocoyam and Melon are popularly used in Southern Eastern Nigeria as soup thickener. Their high carbohydrate content and oil content respectively could increase one's risk of weight gain. On the other hand, *Mucuna sloanei*which could be used as a substitute because of its high protein content is most often neglected. Despite the numerous nutritional and health benefits associated with *Mucuna Sloanei*, the seed has been neglected; hence there is dearth of information on its fungal contamination and biochemical properties particularly on both raw and boiled seeds. This research is therefore aimedat;

1. Comparing the incidence of fungi between raw and boiled seeds of *MucunaSloanei*.
2. Assessing the effect of boiling on the proximate, mineral and anti-nutritive contents of the seeds.

Adequate information on the fungal contamination and proximate composition of these seedsis a prerequisite for its effectiveutilization.

2. MATERIALS ANDMETHODS

2.1 Collection ofSamples

*Mucuna sloanei*seeds (raw and boiled) were purchased from Rumukwurushi Market in Port Harcourt, Nigeria. The samples were taken to the plant pathology laboratory for further studies.

2.2 Proximate Composition Determination

The samples of *Mucuna sloanei*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).

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2.3 MediaPreparation

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.

2.4 Isolation and identification offungi

Five seeds of *Mucuna sloanei* 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).

2.5 Percentage incidence offungi

Incidence of fungi was determined by using the formula:

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

2.6 PathogenicityStudies

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

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

3. RESULTS

Results on the percentage incidence of fungi isolated from raw and boiled samples are presented in table 1.

Table 1: Mean percentage incidence of fungi isolated from Raw and Boiled seeds of *Mucuna sloanei*(Ukpo)

Fungi Isolates	Raw *Mucuna sloanei* (Values %)	Boiled *Mucuna sloanei* (Values %)
Aspergillus niger	50 ± 0.65	60±0.38
Aspergillus flavus	32.8 ± 0.21	30±0.30
Rhizopus stolonifer	48±0.22	52±0.22
Penicillium italicum	-	20±0.50
Fusarium solani	-	80±1.03
Alternaria altermata	-	12.5±0.55

The results revealed *Aspergillus Niger* (50%), *Rhizopus Stolonifer* 48% and *Aspergillus Flavus* (32.8) were isolated from raw *M. sloanei*seeds, while *Fusarium solani* (80%), *Aspergillus niger* (60%), *Rhizopusstolonifer* (52%), *Aspergillus flavus* (30%), *Penicillium italicum* (20%) and *Alternaria altermata* (12.5%) were isolated from boiled seeds of *Mucuna sloanei.Rhizopus stolonifer and Aspergillus* species were predominant in *Mucuna*

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*sloanei*seeds (raw and boiled).Percentage incidence of fungi isolated was higher in boiled samples. (Table 1). *Penicillium Italicum, Fusarium solani* and *Alternaria altermata* were found in boiled samples only. *Fusarium moliniforme* had the highest incidence followed by *Aspergillus niger, Rhizopus stolonifer*, *Aspergillus flavus*, *Penicillium italicum* and *Alternaria altermata*.

Results on proximate composition of raw and boiled *Mucuna sloanei* are presented in Table 2.

Table 2: Proximate composition of Raw and Boiled *Mucuna sloanei*

Parameter	Raw *M. sloanei* (Values %)	Boiled *M. sloanei* (Values %)
Moisture	10.5 ± 0.20	14.6±0.22
Ash	3.3 ± 0.26	3.2±0.26
Fibre	3.8±0.33	3.3±0.21
Lipid	8.3±1.00	8.1±1.60
Carbohydrate	54.5±0.94	49.6±0.91
Protein	19.6±0.11	21.2±0.32

Comparing the values of proximate composition (Moisture, ash, fibre, lipid, carbohydrate and protein) between raw and boiled seed samples of *M. sloanei*. It was observed that boiling decreased the ash, fibre, lipid and carbohydrate contents but increased the moisture and protein contents. (Table 2)

Results on mineral contents and phytochemicals of raw and boiled samples of *M.sloanei* are presented in table 3 and 4

Table 3: Mineral contents of raw and boiled *Mucuna sloanei* (Ukpo)

Parameter	Raw *M. sloanei* (Values %)	Boiled *M. sloanei* (Values %)
Calcium	0.95 ± 0.13	0.92±0.10
Phosphorus	1.5 ± 0.02	1.6±0.32
Sodium	0.05±0.06	0.05±0.08
Potassium	1.50±0.03	1.75±0.15
Iron	0.80±0.06	0.81±0.02
Magnesium	5.12±0.50	5.14±0.55

The mineral contents comprising of Calcium, Phosphorus, Potassium, Iron and Magnesium were also affected by boiling except Sodium. Boiling decreased the value of Calcium but increased Phosphorus, Iron, Potassium and Magnesium.However, boiling did not have any impact on Sodium as the values remain the same before and after boiling as seen in table 3.

Table 4: Phytochemical (Anti-nutritional composition) of raw and boiled *Mucuna sloanei* (Ukpo)

Parameter	Raw *M. sloanei* (Values %)	Boiled *M. Sloanei* (Values %)
Tannin	0.02 ± 0.03	0.01±0.00
Total Oxalate	0.75 ± 0.30	0.32±0.10
Cyanide	0.55±0.22	0.41±0.05

Similar observation was made in the phytochemical composition. Boiling decreased Tannin, total oxalate and Cyanide. (Table 4)

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4. DISCUSSION

4.1 Percentage incidence ofFungi

Results from incidence of fungi as seen in table 1 showed that boiled sample had a higher moisture content which encouraged fungal attack because fungi thrive in moisture.

Thus, the boiled form is more prone to fungal attack. More so, the method of processing, handling and preservation affects the level of contamination and influence of 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 crops (Etebu and Emiri 2016; Ikechi-Nwogu and Chime 2017). Fungi can be found virtually everywhere. It can grow on almost any organic substance as long as moisture and oxygen are present (Ueno, 2000). This result also agrees with Obiakor – Okeke *et al;* (2014) who isolated *Aspergillus* and *Rhizopusspecies* from *M. sloanei*seeds. Gbarabe *et al.,* (2014) also isolated *Aspergillus species, Penicillium species* and *Botryodiplodia species* from the same seed.

Generally, five genera of fungi (*Aspergillus, Rhizopus, Penicillium, Alternaria* and *Fusarium*) were isolated. *Aspergillus* and *Rhizopus* were predominant in both raw and boiled samples. It therefore suggests, that *Rhizopus* and *Aspergillus* species are more abundant in the air/environment than other fungal species.

4.2. ProximateComposition.

As shown in table 2; the moisture content ranged between 10.5% - 14.6%. These values are high and will encourage deterioration due to microbial attack. Boiling increased the moisture content, it was expected because the seeds absorbed moisture (water)during boiling. This is comparable with 10.5% - 12.0% reported by Obiakor – Okeke *et al,* (2014) on the same seed. The moisture content of *M.sloanei*was significantly higher than the reports of Igwenyi and Azoro (2014) on the sameseed.

The ash Content values 3.3-3.2% for raw and boiled samples respectively were very low. Boiling decreased the ash content, though the difference is not significant.This observation agrees with the findings of earlier workers. (Obiakor-Okeke *et al*., 2014; Igwenyi and Azoro 2014).

The percentage fibre also decreased with boiling 3.8-3.3%. The values were higher than 2.99% reported by Igwenyi and Azoro (2014) on the rawseeds of *Mucuna sloanei*. The fibrecontent of raw sample is comparable to 3. 8% reported by Obiakor- Okeke *et al*, (2014) on raw sample of the same seed. However, this result negates the assertion of (Obiakor- Okeke *et al,* 2014) who reported that cooking increased the fiber content of *Mucuna sloanei*. Fiber regulates bowel actions and may help to guard against colon and rectal cancer as well as diabetes. Fiber supplements or fibre – rich foods may function as normal dietary agents by modulating the digestive and absorptive process (Okaka *et al*, 2006) .

The percentage compositions of lipids in the samples were 8.3 -8.1%. It thus suggests that boiling decreased the composition of lipids. These values were lower than 14-18.5% reported by Uhegbu *et al,* (2009) but higher than 6.25% and 6.5% reported by Igwenyi and Azoro (2014) and Obiakor- Okeke *et al*, (2014) respectively on raw samples of the same seed. The values of the lipid content were also higher than the result of Igwenyi and Akubugwo (2010). Obioakor – Okeke *et al.,*(2014) reported that cooking increased the lipid content of *M. sloanei*(6.5 -8.3%) the result from this work negates their assertion. 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.

Result on table 2 revealed that *M.sloanei*had high carbohydrate content of 54.5% -49.6% for raw and boiled samples respectively. It therefore implies that, boiling decreased the carbohydrate content. The decrease could be attributed to the conversion of carbohydrates to simple sugars which is further converted to alcohol and carbon dioxide. This observation did not agree with the report of Obiakor- Okeke *et al.,(*2014) who

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reported an increase in carbohydrate content (54.4-62.3) after cooking. The variations could be as a result of the processing method in the preparation of the seed samples and other environmental factors.

The values were lower than 70.71% reported by Igwenyi and Azoro (2014), and Akubugwo (2010) on raw seeds of *M. sloanei*. The values however, comparable to 54.4% reported by Obiakor – Okeke *et al,* (2014) on raw samples of the same seed.

The carbohydrate contents were also comparable to 57-59% for *Barachystegia eurycoma* and *Detarium microcarpum* (Uhegbu *et al.,* 2009), also used as soup thickeners.

The protein contents of the seeds ranged between 19.6-21.2%. Boiling increased the protein content, which negates the findings of earlier workers on the same seed. (Obiakor – Okeke *et al*., 2014). The protein contents of the seeds were significantly higher than 12.5% reported by Igwenyi and Azoro (2014), but lower than 24% reported by Obiakor – Okeke *et al;* (2014) on raw samples of the same seed. These values give the seeds positive attributes as plant proteins are scarce and this protein can furnish the essential amino-acids needed for healthy growth and repair of tissues (Igwenyi; 2008). The protein content is also lower than 23% – 35% reported by Bressani (2002)

4.3 MineralComposition

From the results presented in table 3; Boiling decreased the content of Calcium but increased Phosphorus, Iron, Potassium and Magnesium contents. Sodium was not affected by boiling. This observation negates the findings of (Obiakor – Okeke *et al;* 2014) who reported that cooking increased the calcium content but decreased Iron and Phosphorus contents of *M. sloanei*.

The variations could be attributed to processing methods.

4.4 Phytochemicals(Anti-nutritionalComposition):

Boiling decreased the phytochemicals comprising of Tannin, Total Oxalate and Cyanide (Table 4). This is in line with the result of Obiakor – Okeke *et al;* (2014).

This also agrees with the findings of Enwere (1998) which posits that some legumes are made edible by extensive hydrolysis of their indigestible components and elimination of nutritional stress factors such as anti-nutritional factors and toxic components through the action of fermenting microorganisms during fermentation. Tannins are known to possess health benefits, wherein they are 15 – 30 times more efficient in free radical quenching activity than trolox and other simple phenolics (Hurnel *et al;* 1999).

CONCLUSION

Boiling increased the moisture contents of *M. sloanei* (Ukpo), consequently encouraged deterioration due to microbial (fungi) attack.

It also increased the protein content but decreased Ash, fiber, lipid and carbohydrate. Boiling also affected the mineral content; it decreased calcium, but increased Phosphorus, Potassium, Iron and Magnesium. *M. sloanei*seed has an appreciable percentage yield of carbohydrate that serves both as thickener and fuel source for the generation of energy currency of thecell.

The protein contents showed that they can provide the amino acids needed to support the metabolic activities of the body. Boiled samples haboured more fungi species than raw samples. It therefore suggests that boiled Ukpo seeds 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 associateddiseases.

However, effect of mycodeterioration on the proximate compositions of *M. sloanei*is advocated.

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