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Comparative Studies on Proximate, Mineral and Mycological Analysis of Coated and Decoated Seeds of *Citrullus colocynthis* (Melon)

Dr. Emiri U. N.
Senior Lecturer, Department of Agricultural Education,
Isaac Jasper Boro College of Education, Bayelsa State, Nigeria
Disegha G. C.
Lecturer, Department of Microbiology, Rivers State University, Port Harcourt, Nigeria

Abstract:
Comparative studies on proximate, mineral and mycological analysis of coated and decoated seeds of Citrullus colocynthis were investigated. The results revealed that the seed was predominantly composed of lipids and carbohydrates. The lipid contents were 37.2 and 41.6% for coated and decoated seeds respectively. The protein contents for coated and decoated seeds were 30.45 and 25.4% respectively. The concentration of moisture, Ash, Fibre and Carbohydrates were (10.5 and 13.4%), (4.5 and 5.7%), (8.75 and 2.5%), (8.56 and 11.25%) respectively. The concentration of Magnesium, Phosphorus and Calcium were relatively high when compared to Potassium, Iron and Sodium. All the mineral contents analyzed were relatively higher in coated samples except iron. Five genera of fungi (Rhizopus, Aspergillus, Penicillium, Sclerotium and Alternaria) were isolated from melon seeds Rhizopus stolonifer 60%, Aspergillus niger 42%, Aspergillus flavus 25% were isolated from coated seeds. While from decoated seeds R. stolonifer 50%, A. niger 60%, and A. flavus 40% were isolated. Decoated seeds harboured more fungi species.

Keyword: *Citrullus colocynthis, seeds, coated, decoated, fungi, proximate composition*

1. Introduction

Citrullus colocynthis (melon is a creeping annual belonging to the Cucurbitacea family (Obute and Ndukwu 2005; Odiaka and Schippers, 2004). The melon plant has smooth spherical fruits of the size of cucumber sometimes or as big as a small ball. The fruit is green when young and somewhat yellow when ripe and contains a soft spongy pulp in which as embedded numerous oval-shaped, compressed, white or brown seeds (Ude *et al*., 2002).

C. colocynthis seeds are high in protein (34.86%), oil (42.29%) minerals such as sodium (162.76ppm), potassium (8.28%), Calcium (1.49%), Copper (3.37ppm) and Zinc (13.46ppm) (Abiodun and Adeleke, 2010). Thus *C. colocynthis* is a major source of protein in the tropical regions of Africa and Asia where staples are very low in protein. Due to its high protein content, it is used as food condiment and soup thickener. The seeds are roasted and ground into a coarse, nutritional meal which is a local staple in Kalahari regions of Africa. Also in Sudan and Egypt *C. colocynthis* seeds are roasted and the pulp is eaten as a dish called 'tasali' (Van Dersvossen *et al.,* 2004).

In Nigeria, *C.colocynthis*seeds are fermented and eaten as "Ogiri" (Abiodun *et al.,* 2010). The seeds can also be roasted, pounded, fried and then boiled to prepare a sweater known as 'Igbalo' (Van der vossen *et al.,* 2004).

C. colocynthis seeds, however have a challenge of fieldand storage fungal contamination. Many researchers have reported storage fungal pathogen. Bankole *et al.,* (2005) reported the presence of Aflatoxins in *C. colocynthis* grown in Nigeria. Information on the pathology of coated and decoated seeds of *C. colocynthis* and their chemical analysis is lacking hence this research was aimed at isolating seed-borne fungi associated with *C.colocynthis* and investigating their proximate and mineral compositions.

2. Materials and Methods

2.1. Collection of Samples

Coated and decoated *C.colocynthis* (Melon) 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.

2.2. Proximate Composition Determination

The samples of *C. colocynthis* 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).

2.3. 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.

2.4. Mycological Studies

2.4.1. Isolation and identification of Fungi

Ten seeds of *C. colocynthis* 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 growing from the seeds were later identified using fungi identification guides by Barnett and Hunter (1999) and Alexopoulous *et al.,* (2002).

2.4.2. Pathogenicity Studies

Healthy seeds samples of *C. colocynthis* 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 C. *colocynthis* seeds 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.

2.4.3. Mean percentage Incidence of Fungi

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

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

3.Results

Results on the percentage incidence of fungi isolated from coated and decoated seeds *C. colocynthis* are presented in table 1, proximate and mineral compositions are presented in Table 2 and 3 respectively. Results on phytochemicals are presented in Table 4.

Fungal Isolates	Coated *C. colocynthis* (Values %)	Decoated *C. colocynthis* (Melon) (Values %)
Rhizopus stolonifer	60 ± 0.38	50 ± 0.45
Aspergillus niger	42 ± 0.22	68 ± 0.55
Aspergillus flavus	25 ± 0.50	40 ± 0.32
Aspergillus nidulans	-	30 ± 0.32
Penicillium italicum	-	10 ± 0.20
Sclerotium rolfsii	-	20 ± 0.22
Alternaria altermata	18 ± 0.55	-

Table 1: Mean percentage incidence of fungi isolated from coated and decoated C. colocynthis

Results revealed that *Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* were predominant in *C. colocynthis* seeds (coated and decoated). Percentage incidence of fungi isolated was higher in decoated samples as shown in Table 1. However, *Alternaria altermata* was not found in decoated sample but was found in coated samples, while *Apergillus nidulans, Penicillium italicum* and *Sclerotium rolfsii* were not found in coated samples but were found in decoated samples.

Parameter	Coated *C. colocynthis* (Values %)	Decoated *C. colocynthis* (Melon) (Values %)
Moisture	10.5 ± 0.20	13.4 ± 0.32
Ash	4.5 ± 0.22	5.7 ± 0.30
Fibre	8.75 ± 0.50	2.5 0.20
Lipid	37.2 ± 0.94	41.6 ± 0.91
Carbohydrate	8.56 ± 0.52	11.25 ± 0.22
Protein	30.45 ± 0.36	25.4 ± 0.32

Table 2: Proximate composition of coated and decoated C. colocynthis seeds

Results showed that the composition of Moisture, Ash, Lipid and Carbohydrate were higher in decoated samples while Fibre and Protein were higher in coated samples. Lipid and protein were predominantly higher in both samples.

Parameter	Coated *C. colocynthis* (Values %)	Decoated *C. colocynthis* (Melon) (Values %)
Calcium	2.15 ± 0.21	1.17 ± 0.22
Phosphorus	3.6 ± 0.42	3.1 ± 0.332
Sodium	0.12 ± 0.01	0.04 ± 0.03
Potassium	1.35 ± 0.20	0.68 ± 0.15
Iron	0.70 ± 0.08	0.75 ± 0.06
Magnesium	4.5 ± 0.45	4.4 ± 0.48

Table 3: Mineral Content of coated and decoated C. colocynthis seeds

Results revealed that mineral composition comprising of Calcium, Phosphorus, Sodium, Potassium and Magnesium were higher in decoated sample. (Table 3)

Parameter	Coated *C. colocynthis* (Values %)	Decoated *C. colocynthis* (Melon) (Values %)
Tannin	1.00	0.81
Total Oxalate	0.14	0.15
Hydrogen Cyanide	0.00	0.00

Table 4: Phytochemical of coated and decoated C. colocynthis seeds

Results showed that Tannin was predominant in Melon seeds. However, there was no significant difference in values of the phytochemicals assessed in both samples. (Table 4).

4.Discussion

4.1. Percentage Incidence of Fungi in Melon Seeds

Generally, five genera of fungi were isolated from coated and decoated *C.colocynthis* seeds; *Rhizopus, Aspergillus, Penicillium, Sclerotium and Alternaria. Aspergillus niger* recorded the highest percentage incidence while the least was recorded by *Penicillium italicum.* This is in agreement with the report of Bankole *et al.,* (2005) which posits that stored *C.colocynthis*harbor many species of *Aspergillus* and a few of *Penicillium.*

Many researchers have reported similar fungal pathogens as isolates of *C.colocynthis* grown in Nigeria. (Bankole and Joda, Chiejina, 2006; Kehine, 2011; Nwokocha and Opara, 2016).

Coated melon seeds harbored fewer fungi compared to decoated seeds. Whilst, three genera of fungi (*Rizopus, Aspergillus* and *Alternaria spp*) were isolated from coated seeds, four genera of fungi ((*Rizopus, Aspergillus, Penicillium* and *Sclerotium spp*) were isolated from decoated seeds. This is because the seeds are protected by differential integumentary structure (Seed coat) which serves as a barrier to microbial invasion; hence decoated melon seeds had more fungal attack. This could also be attributed to post processing methods and preservation.

4.2. Proximate Composition of Melon Seeds

The moisture contents were 10.5 and 13.4% respectively for coated and decoated *C. colocynthis* respectively. (Table 1) These values were significantly higher than 4.4% moisture reported by Igweny *et al.,* (2011) on the same seed. The moisture contents were higher and will encourage deterioration due to microbial attack. The value of moisture content of coated sample was lower compared to decoated samples. This was anticipated given the hard and dry nature of the seed coats. Although the water content of a food is expressed as a percent, this number does not reflect how the water exists in the food. Water in the food is classified according to its availability or biological activity and is either “free” or “bound”.

The Ash contents were 4.5 and 5.7% for coated and decoated samples respectively. The values were higher than 2.86% reported by Igwenyi *et al.,* (2011) on the same seed. The increase could be attributed to the processing method in the preparation of the seed samples and other environmental factors.

The fibre content was higher in coated sample (8.75%) than decoated sample (2.5%). These values were higher than the values reported by Igwenyi *et al.,* (2011) on *C. colocynthis* seeds but comparable to 8.53% reported by Igwenyi *et al.,* (2011) on *Irvingia gabonensis* which is also used as soup thickener. Fibre supplements or fibre-rich foods may function as normal dietary agents by modulating the digestive and absorptive process (Okaka *et al.,* 2006).

The lipid contents were high ranging from 37.2 – 41.6% for coated and decoated samples respectively. It thus suggests that melon seeds are rich in oil. This result agrees with the assertion of Abiodun and Adeleke (2010) who reported 42.29% lipid on melon seed. It however negates the assertion of Igwenyi *et al.,* (2011) who reported 20.70% lipid on the same seed. The oil contents were however lower than 59.46% as reported for *cucumis melo var. agretis scrab* seeds in Nigeria (Adekunle and Olumo, 2008).

The percentage carbohydrate compositions were low; 8.56 and 11.25% for coated and decoated seeds respectively. Decoated seed had higher carbohydrate content than coated seeds. The values were significantly lower 70.17% as reported for the same seed. (Igwenyi *et al.,* 2011). The low carbohydrate value is an indication of the fact that *C. colocynthis* seed is poor in carbohydrate. The protein content was higher (30.45%) in coated seeds compared to decoated seeds (25.4%). The values suggest that melon seed is a good source of plant protein. These values are comparable to 34.86% as reported for the same seed (Abiodun and Adeleke 2010) but higher than the values reported by Igwenyi *et al.,* (2011).

4.3. Mineral Contents of Coated and decoated C. colocynthis

The mineral analysis revealed that melon seeds are not rich in mineral composition as indicated by the percentage Calcium, Sodium, Potassium, Iron and Phosphorus. The values were lower in decoated seeds compared to coated seeds. The values of calcium 2.15 and 1.17% for coated and decoated seeds respectively are comparable to 1.49% reported by earlier workers (Abiodun and Adeleke, 2010). The values of Potassium 1.35 and 0.68% for coated and decoated samples respectively were significantly lower than 8.28% reported for the same seed. (Abiodun and Adeleke 2010). Minerals are essential elements that exist in non-organic form and are normally required in small amounts hence they, like vitamins are tagged micronutrients (Underwood, 1997). They are essential to life and an element is said to be essential when a deficiency in intake produces an impairment of function and physiological amounts of only that element can prevent or alleviate the impairment. According to Boukari *et al.,* (2001), calcium intake is very low in developing countries, far below the recommended daily allowance for adults. Calcium plays a role in supportive structures of the body and its dietary deficiency together with phosphorus and vitamin D causes rickets in children, osteoporosis and osteomalacia in adults. Inorganic phosphates are necessary in the generation of the energy currency of the body (ATP). (Voet and Voet, 2004).

4.4. Phytochemicals of coated and decoated C. colocynthis

Results showed that phytochemicals analysis comprising of Tannin, oxalate and hydrogen cyanide were very low.

Tannins are known to possess health benefits, wherein they are 15 – 30 times more efficient in free radical quenching activity than trohox and other simple phenolic (Hurnel *et al.,*1999). They have also been shown to play very significant roles in human medicine and treatment of ailments. (Addae – Mensah, 1992).

C. colocynthis seed does not contain hydrogen cyanide as revealed in the results. (Table 4)

5.Conclusion

C. colocynthis seeds are rich in protein. The protein contents showed that they can provide the amino acids needed to support the metabolic activities of the body. The oil content suggests that the seed is an oil seed and can serve as commercial source of vegetable oil. The moisture value was high particularly in decoated samples and will encourage deterioration due to microbial attack. Hence decoated seed samples harboured more fungi. Storage will entail a careful reduction in moisture to discourage microbial growth and deterioration.

However, the effect of mycodetoriation on the proximate composition of *C. colocynthis* is advocated.

6.References

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